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ERRATA AND AUTHOR'S EMENDATIONS

Page 61, footnote to table 5, "26° for hours" should be "26° for 8 hours."

Page 69, author's name "Matthias" should be "Mathias."

Page 74, paragraph 1, line 2, "high humidity" should be "low humidity."

Page 80, paragraph 2, line 1, "haustoria" should be "mycelia."

Page 97, line 17, " $\frac{\sigma=A_s}{v}$ " should be " $\sigma=\frac{A_s}{v}$."

Page 97, equation (10), " C_p " should be " \bar{C}_p ."

Page 98, line 24, " $\frac{4}{\sigma}$ " should be " $\frac{4}{\sigma}$."

Page 104, equation 30, the denominator should read

$$C_7 \gamma \bar{C}_{cp} \bar{C}_v k n \left[C_i \left(\frac{t_f - t_o}{t_f - t_i} \right) \right].$$

Page 170, paragraph 1, line 3, "(9)" should be "(8)."

Page 176, figure 2, legend, line 4, "R19" should be "K19."

Page 182, table 16, first column under "Pollinated" should be headed by "Micrograms per gram."

Page 291, paragraph 1, line 10, "e" should be " e^{-1} ".

Page 291, line 5, should read

$$1 - \left(\frac{n}{N} \frac{N}{n} \right) + \frac{\frac{N}{n} \left(\frac{N}{n} - 1 \right) \left(\frac{n}{N} \right)^2}{2} - \frac{\frac{N}{n} \left(\frac{N}{n} - 1 \right) \left(\frac{N}{n} \right) \left(\frac{n}{N} \right)^3}{2 \cdot 3} + \dots$$

Page 306, paragraph 1, line 8, "27 percent" should be "47 percent."

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No. 1

INFLUENCE OF LIGHT AND TEMPERATURE ON SUGAR-CANE AND *ERIANTHUS*¹

By JOHN I. LAURITZEN, *senior physiologist*, E. W. BRANDES, *head pathologist in charge*, and JULIUS MATZ, *pathologist*, *Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In a previous publication (2, p. 3),² dealing with the photothermal requirements of sugarcane, evidence was submitted that "at given temperatures there are definite light requirements, the light needs being greater at the higher temperatures and less at lower temperatures." For example, at 59° F., when three 40-watt fluorescent daylight tubes and two 100-watt Mazda bulbs were used as the source of light, the plants showed slight growth and continued in fairly normal health for 8 weeks; whereas, with the same light source at 78°, there was little growth and marked impairment of health, death resulting in many cases. At 59° the anabolic and catabolic processes of the plants were in approximate balance, whereas at 78° the catabolic processes exceeded the anabolic, especially during the later weeks of the experiment. As the light intensity at 78° was increased, there was a marked improvement in the health and growth of the plants. The existence of a fundamental relation between the factors of light and temperature in their effects upon the development of plants was revealed by the writers in these earlier experiments and led to the further studies reported in the present paper. Bolas (1) found a similar relation between temperature and light intensity and assimilation rate (measured by change in dry weight) in tomato plants during a period of 7 hours.

The investigations reported in this paper and in a previous one (2) included (1) studies on the rate of growth of a group of wild and garden varieties of sugarcane, indigenous to different latitudes, that were assembled and studied individually at a series of stations at various locations extending from the Equator to latitude 40° N., and (2) rate-of-growth studies of some of these same varieties as well as additional ones under controlled conditions of temperature and light. From November 19, 1940, to July 11, 1941, five experiments were conducted at the United States Department of Agriculture Cold Storage Laboratory, Arlington Experiment Farm, Arlington, Va.

MATERIALS AND METHODS

The plants used in the present studies included 3 varieties of *erianthus* and 21 varieties of sugarcane, 8 of which had been used in the rate-of-growth studies (table 1). The experimental environmental conditions included additional temperatures and a wider range of

¹ Received for publication March 22, 1944.

² Italic numbers in parentheses refer to Literature Cited, p. 18.

light intensities than was previously employed. The plant materials for the most part were selected with consideration for the significance of latitude in growth and light-temperature relations, and selection was limited to forms known with reasonable certainty to have originated at particular latitudes.

TABLE 1.—Statistics on varieties of sugarcane and erianthus studied

Species and variety ¹	Wild or garden	Importation No.	Place of origin	Latitude where indigenous
<i>Erianthus arundinaceus</i> (Retz.) Jesw.: 28 N.G. 7.	Wild	631	Territory of Papua, New Guinea	8° S.
<i>Erianthus marimus</i> Brongn.: N.C. 132 ²	do	921	New Caledonia, French Oceania	21° S.
Raiatea ²	do	923	Society Islands, French Oceania	17° S.
<i>Saccharum barberi</i> Jesw.: Hatooni	Garden	213	British India	25° N.
<i>Saccharum officinarum</i> L.: Caña Criolla ²	do	615	do	11° N.
Louisiana Purple	do	5	Java, Dutch East Indies	6° S.
N.C. 25 ²	do	888	New Caledonia, French Oceania	21° S.
N.C. 117	do	909	do	21° S.
" <i>Saccharum robustum</i> ": N.H. 1	Wild	933	New Hebrides, South Pacific	17° S.
<i>Saccharum sinense</i> Roxb.: Cayana ²	Garden	126	British India	31° N.
<i>Saccharum spontaneum</i> L.: Burma ²	Wild	848	do	20° N.
Djatiroto	do	569	Java, Dutch East Indies	8° S.
Gehra Bon	do	618	British India	25° N.
Paseroean	do	555	Java, Dutch East Indies	8° S.
Rellagadi	do	617	British India	19° N.
Tabongo ²	do	578	North Celebes, Dutch East Indies	0°
U.S. 4515 ²	do		Turkmenistan, Union of Soviet Socialist Republics.	40° N.
<i>Saccharum</i> hybrids: ³ Co. 281	Garden	176	Coimbatore, British India	11° N.
C.P. 1165	(⁴)		Canal Point, Fla.	27° N.
C.P. 29/291	(⁴)		do	27° N.
C.P. 31/204	(⁴)		do	27° N.
C.P. 31/511	Garden		do	27° N.
C.P. 34/79	(⁴)		do	27° N.
P.O.J. 2725	Garden		Java, Dutch East Indies	8° S.

¹ Varieties of sugarcane are often designated by letters or other abbreviations that usually indicate place or institution of origin. The meanings of designations used in this paper are as follows: Co., Coimbatore (India); C.P., Canal Point (Fla.); N.C., New Caledonia; N.G., New Guinea; N.H., New Hebrides; P.O.J., Preoetation Oost Java; and U.S., United States.

² Used in rate-of-growth studies.

³ The hybrids were derived from both wild and garden stock of sugarcane.

⁴ Bred at Canal Point, Fla., but not released for commercial cultivation.

Nine insulated rooms (eight of them approximately 7 by 14 feet by 11 feet high and one approximately 8 by 9 feet by 8 feet high) provided space for the controlled conditions (one condition of light intensity to each room) during the first three experiments; seven of the larger rooms provided space during the fourth experiment. The control rooms were cooled by brine coils, and the temperature was maintained within 4° F. (mostly within less than 2°) by the use of thermostatically controlled electric heaters.

Air exchange was provided in each room by forcing air through a 2-inch duct that led to the rear of the room. A similar duct through the front wall permitted escape of the air. The air was exchanged about once every hour. The relative humidity in the rooms varied from about 60 to 80 percent, the percentage tending to increase with lowering of the temperature.

The light source in each room was a combination of Mazda lamps and daylight fluorescent tubes. In eight of the nine rooms used the lights were mounted on a frame attached to pulleys for raising or lowering the light source to the desired height (1 foot) above the tops

of the plants. The lights in each room were arranged so as to distribute the light at as uniform intensity as possible over an area of 24 square feet (4 by 6 feet), the space occupied by the plants. In the ninth room the lights were stationary at about 4 to 5 feet above the tops of the plants. This room was the source of the lowest light intensity employed and was used only in connection with temperatures of 60° and 65° F. In none of the rooms was the light intensity uniform over the entire area occupied by the plants.

This variation in intensity was influenced particularly by the position of the Mazda lamps. The approximate intensities corresponding to the number and combinations of Mazda and fluorescent lights used in experiments 1 to 4 are shown in table 2. It should be kept in mind that the light intensities employed in all these experiments were relatively low as compared with normal outside light. In all experiments the daily light and dark periods were 13 and 11 hours, respectively.

TABLE 2.—*Source and approximate intensities of light used in experiments 1 to 4*

[Some of the intensities were duplicated at the different temperatures employed]

Light source					Approximate intensity 1 foot from source ¹
Fluorescent tubes			Mazda bulbs		
Total	Length	Power	Total	Power	
<i>Number</i>	<i>Inches</i>	<i>Watts</i>	<i>Number</i>	<i>Watts</i>	<i>Foot-candles</i>
1	24	20	1	50	20
2	48	40	1	100	50
4	48	40	2	100	100
9	48	40	4	100	220
18	48	40	8	100	340
35	48	40	16	100	500

¹ The source for the lowest light intensity used (20 foot-candles) was about 4 to 5 feet above the plants.

The source of light in experiment 5 was water-cooled, 1,000-watt Mazda lamps suspended, by a pulley arrangement for altering the height of the light, in chambers (18 inches square and 12 feet high) that were provided with air exchange. These lamps furnished an intensity of about 1,750 foot-candles at the top of the plants. The 200-foot-candle intensity was obtained by covering the lights with paper or muslin. The light intensities were measured 1 foot below the light source. In experiments 1 to 4 there was one light intensity (100 foot-candles) common to all temperatures. In the first three experiments there was one additional intensity (50 foot-candles) common to the two lower temperatures (60° and 70° F.) and one additional intensity (220 foot-candles) common to the two higher temperatures (70° and 78° in experiment 1, and 70° and 86° in experiments 2 and 3).

In addition to growing the different varieties of sugarcane and erianthus under the foregoing conditions of light and temperature, in two of the experiments control plants were grown in the greenhouse.

The plants were grown from stem cuttings in 4-inch pots in a greenhouse. About 10 to 14 days before the plants were exposed to the various conditions of light and temperature they were transferred to 6-inch pots. The cuttings were planted and the plants exposed to experimental conditions of temperature and light on the following

dates: Experiment 1, August 20 and November 19, 1940; experiments 2 and 5, October 8, 1940, and January 10, 1941; experiment 3, January 29 and March 25, 1941; and experiment 4, April 10 and June 13, 1941. The age of the plants at the beginning of the experiments was as follows: Experiment 1, 91 days; experiments 2 and 5, 94 days; experiment 3, 55 days; and experiment 4, 64 days.

Plants of a given variety in a given experiment were fairly uniform in size, but the sizes differed considerably between varieties, especially in the experiments in which the older plants were used. When dealing with a collection of varieties such as those used in these experiments, it is impossible, although highly desirable, to obtain plants of different

The diagram shows a rectangular room measuring 4 feet in width and 6 feet in height. The room is divided into a 4x6 grid of 24 individual plant positions. Each position is labeled with a variety name. The varieties are distributed as follows:

RELLAGADI	N.C.132	LOUISIANA PURPLE	TABONGO
N.H.1	RAIATEA	RELLAGADI	N.C.25
CAYANA	LOUISIANA PURPLE	HATOONI	RAIATEA
N.C.132	RAIATEA	TABONGO	N.H.1
RELLAGADI	N.C.25	CAYANA	LOUISIANA PURPLE
HATOONI	N.H.1	N.C.132	TABONGO
RAIATEA	HATOONI	N.C.25	CAYANA

FIGURE 1.-- Distribution in each room, beneath the source of light, of individual plants of the different varieties of sugarcane and erianthus used in experiment 3. Similar distributions were made in experiments 1, 2, and 4.

varieties of uniform size. The procedure followed was to grow the plants under uniform conditions and then select within a given variety for uniformity.

In order to compensate somewhat for the variation in light intensity at different points under the batteries of lights, the plants of a given variety were distributed in the same manner in each room (fig. 1).

The number of plants used at each condition of light and temperature in experiments 1 to 4 was 24, 32, 28, and 32, respectively. In most instances 2 plants of each variety were used in experiment 1, and 3 plants of each variety in each of experiments 2, 3, and 4 (see figs. 2 to 5). In each room the plants were arranged in a rectangular area (4 by 6 feet) directly under the lights, with 4 rows one

way and 6, 7, or 8 rows the other, depending upon the number of plants used. The pots containing the plants rested on an open rack, 6 inches above the floor. Daily attention was given to the water requirements of the plants; and, although the soil was not maintained at a constant water content, an effort was made to approximate the same water content in all pots. A record was kept of the amount of water added.

In experiment 5 only one plant of each of two varieties was exposed to each of two light intensities.

Weekly measurements of the height of the plants were taken, and observations were made on the color, health, and death of the leaves and on death of the plants. At the completion of the experiments, dry-weight determinations were made under comparable conditions of drying (floor of attic in the headhouse of a greenhouse).

EXPERIMENTAL DATA

The data here reported deal with species and varieties of two closely related genera, *Saccharum* and *Erianthus* (table 1). Each is vegetatively propagated and is commonly regarded and designated as a variety. In the illustrations and tables dealing with the response of the plants to light and temperature, the varieties are listed alphabetically, regardless of specific and generic connections. Because of the common response of all varieties, the discussion, in the main, will deal with the response of the varieties as a group.

TABLE 3.—Total average increase in height per plant of mother stalks plus suckers and of mother stalks alone in different varieties of sugarcane and erianthus exposed for 7 weeks to various conditions of light and temperature in 3 experiments¹

MOTHER STALKS PLUS SUCKERS

Experiment No. and variety	Average initial height of mother stalk	Increase in height with indicated light intensity (foot-candles) at --									Green- house con- trols
		60° F.			70° F.			78° or 86° F. ²			
		20	50	100	50	100	220	100	220	340	
Experiment 1:	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>
Burma	117.0	16.0	18.8	36.5	33.8	39.8	80.5	36.5	63.8	131.8	
Cayana	39.5	.5	1.3	3.2	1.8	6.2	18.0	4.3	14.7	47.7	
Gebra Bon	48.0	2.0	7.3	17.3	5.0	16.3	34.0	15.3	13.5	36.5	
Hatooni	36.5	1.0	1.3	4.0	1.8	4.0	9.3	2.8	6.0	25.8	
Louisiana Purple	24.0		.3	.3	.3	0	2.0	.5	1.5	7.0	
N. C. 132	39.0		6.0	9.5	5.5	9.8	22.3	5.8	30.0	39.0	
N. H. 1	63.0	1.3	7.0	16.3	10.3	26.3	42.3	24.0	51.3	63.0	
Paseroean	74.0	1.8	11.5	22.8	11.3	19.3	36.8	28.0	103.0	104.8	
Rellagadi	84.5	23.8	46.8	69.3	24.5	53.3	74.5	20.5	79.8	146.5	
Tabongo	76.0	5.8	21.3	18.7	19.8	48.7	80.8	47.7	91.8	136.8	
28 N.G. 7	27.5	0	.5	0	.8	1.0	19.0	1.0	10.3	29.0	
Total		52.2	122.1	197.9	114.9	224.7	428.5	186.4	465.7	707.9	
Experiment 2:											
Burma	37.1	2.5	15.3	14.5	5.8	10.8	23.8	3.0	29.0	61.8	
Cayana	24.3	.5	1.2	4.2	2.3	7.7	7.7	8.8	13.7	39.8	
Hatooni	22.4	.5	1.5	1.3	.8	1.5	4.7	.8	15.3	12.2	
Louisiana Purple	15.4	.3	1.5	0	.3	1.0	.7	.7	3.0	4.8	
N. C. 25	14.7	.3	.2	.7	.8	.2	1.7	.3	7.7	6.2	
N. C. 132	27.0	1.2	7.5	11.3	10.8	11.0	18.8	11.0	31.0	35.8	
N. H. 1	25.9	3.2	6.8	12.0	8.5	17.0	25.2	7.5	42.0	46.5	
Paseroean	47.9	1.7	7.0	13.8	11.0	20.7	42.5	17.5	72.0	80.2	
Ralatea	21.9	1.2	1.5	4.0	4.0	11.7	21.7	3.2	30.8	53.2	
Tabongo	53.7	3.2	9.5	15.5	17.8	28.0	43.8	30.3	78.5	109.5	
U.S. 4515	39.9	.2	2.3	.7	1.3	.5	5.2	1.0	1.2	3.8	
Total		14.8	53.3	78.0	63.4	105.1	195.8	84.1	324.2	462.8	

See footnotes at end of table.

TABLE 3.—Total average increase in height per plant of mother stalks plus suckers and of mother stalks alone in different varieties of sugarcane and erianthus exposed for 7 weeks to various conditions of light and temperature in 3 experiments ¹—Con.

MOTHER STALKS PLUS SUCKERS—Continued

Experiment No. and variety	Average initial height of mother stalk	Increase in height with indicated light intensity (foot-candles) at—									Green- house con- trols
		60° F.			70° F.			78° or 86° F. ²			
		20	50	100	50	100	220	100	220	340	
Experiment 3:	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>
Cayana	14.1	0	0.3	0.5	0.3	0.8	6.7	0.7	15.0	24.0	44.3
Hatooni	19.8	.3	.8	1.2	.3	1.3	6.5	.7	8.3	17.5	54.8
Louisiana Purple	12.2	.3	.5	.8	.3	.8	5.7	.5	3.5	11.2	14.5
N.C. 25	10.9	0	.5	.5	.5	1.2	3.3	2.2	5.5	10.3	29.5
N.C. 132	10.2	.8	.8	1.8	1.3	5.2	12.3	4.7	17.5	24.0	33.5
N.H. 1	12.5	.3	1.3	1.0	0	3.7	24.0	.5	12.5	39.3	55.0
Raiatea	13.5	.4	.9	3.5	.6	.9	14.8	.9	17.4	25.4	
Rellagadi	10.9	1.0	2.0	6.8	3.3	6.3	30.8	4.0	7.2	32.5	172.2
Tabongo	11.3	1.2	3.1	7.7	2.5	7.3	24.3	4.0	22.8	62.2	99.3
Total		4.3	10.2	23.8	9.1	27.5	128.4	18.2	109.7	246.4	503.1

MOTHER STALKS ALONE

Experiment 1:											
Burma	117.0	14.8	15.3	34.5	30.0	35.3	52.3	28.0	55.0	122.8	
Cayana	39.5	.5	.8	3.0	1.8	6.2	13.3	5.3	13.0	40.0	
Gehra Bon	48.0	.5	2.8	10.5	2.5	8.0	14.3	6.8	4.5	12.0	
Hatooni	36.5	.6	1.0	2.3	1.3	2.0	4.0	2.3	3.8	10.0	
Louisiana Purple	24.0		.3	.3	.3	0	2.0	.5	1.5	7.0	
N.C. 132	39.0		4.8	9.5	5.5	9.8	22.3	5.8	30.0	39.0	
N.H. 1	63.0	1.2	7.0	16.3	10.3	26.0	42.3	24.0	51.3	63.0	
Paserocean	74.0	1.3	11.0	22.5	11.0	18.3	33.8	26.3	102.0	97.0	
Rellagadi	84.5	5.2	8.0	13.3	13.8	22.8	23.0	7.0	47.8	39.8	
Tabongo	70.0	3.5	7.0	8.5	11.3	26.0	37.0	23.7	64.5	58.5	
28 N.G. 7	27.5	0	.5	0	.8	1.0	19.0	1.0	10.3	29.0	
Total		27.6	58.5	120.7	88.6	155.4	263.3	130.7	383.7	518.1	
Experiment 2:											
Burma	37.1	1.3	13.0	11.8	5.3	10.9	23.8	3.0	6.0	46.5	
Cayana	24.3	.5	1.2	1.8	1.7	1.7	5.8	1.0	4.5	28.2	
Hatooni	22.4	.5	1.5	1.3	.8	1.0	4.7	.8	2.7	12.2	
Louisiana Purple	15.4	.3	1.5	0	.3	1.0	.7	.7	3.0	4.8	
N.C. 25	14.7	.3	.2	.7	.8	.2	1.7	.3	4.0	6.2	
N.C. 132	27.0	1.2	7.5	11.3	10.8	11.0	18.8	7.3	28.8	32.3	
N.H. 1	35.9	3.2	5.8	12.0	8.5	17.0	22.5	4.8	36.8	46.2	
Paserocean	47.9	1.7	7.0	13.2	10.5	17.3	38.5	7.3	60.2	69.3	
Raiatea	21.9	1.2	1.3	3.7	2.8	7.8	16.3	3.2	29.8	33.3	
Tabongo	53.7	1.8	4.7	7.8	8.3	17.7	20.2	12.2	28.2	47.7	
U.S. 4515	39.9	.2	.5	0	.2	.3	2.8	.3	1.0	.8	
Total		12.2	44.2	63.6	50.0	85.9	155.8	40.9	205.0	327.5	
Experiment 3:											
Cayana	14.1	0	.3	.3	.3	.7	6.7	.7	3.5	21.2	17.8
Hatooni	19.8	.3	.5	1.2	.3	1.0	6.5	.8	3.7	17.5	14.5
Louisiana Purple	12.2	.2	.5	.8	.3	.8	5.7	.7	3.5	11.2	14.5
N.C. 25	10.9	0	.5	.5	.5	.7	3.3	2.2	5.7	10.0	11.5
N.C. 132	10.2	.8	.8	1.8	1.3	5.2	12.3	1.7	17.5	23.8	16.8
N.H. 1	12.5	.3	1.3	1.0	0	3.7	24.0	.5	12.5	39.3	51.3
Raiatea	13.5	.1	.6	.8	.3	.9	12.0	.6	17.1	25.4	
Rellagadi	10.9	.7	1.0	3.7	1.2	3.0	17.8	1.3	4.0	22.2	33.5
Tabongo	11.3	.3	2.0	5.8	2.2	5.5	15.7	2.3	20.8	50.0	36.0
Total		2.7	7.5	15.9	6.4	21.5	104.0	10.8	88.3	220.6	195.9

¹ The experiments were conducted during the following periods: Experiment 1, Nov. 10, 1940, to Jan. 7, 1941; experiment 2, Jan. 10 to Feb. 28, 1941; and experiment 3, Mar. 25 to May 13, 1941.² The temperature was 78° during experiment 1 and 86° during experiments 2 and 3.

The behavior of the three varieties of *Erianthus* (28 N.G. 7, N.C. 132, and Raiatea) at the various temperatures and light intensities was sufficiently similar to that of certain varieties of *Saccharum* (Gehra Bon, Hattoni, and N.H. 1) to make it impracticable to separate them on a generic basis (table 3). The data do not clearly indicate a distinction between varieties of the different species of *Saccharum*, although the light intensities employed may have been more depressing in their effect on the varieties of *Saccharum officinarum* as a group than on the varieties of the other species of *Saccharum*. Large differences between varieties of *Saccharum*

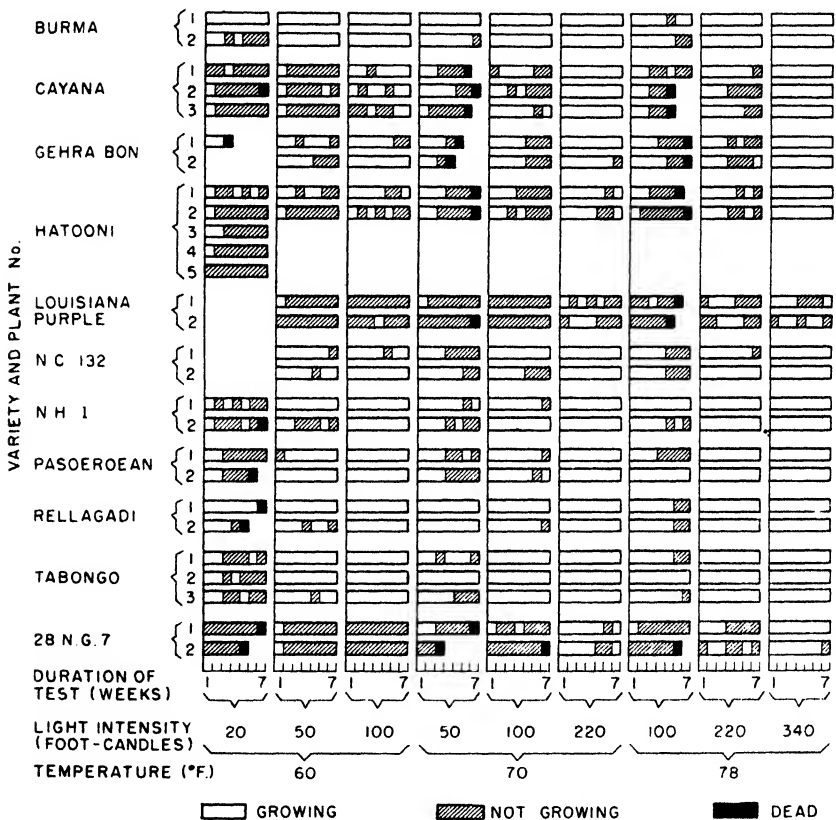


FIGURE 2.—Response of different varieties of sugarcane and erianthus to light intensities at different temperatures in experiment 1. Two plants of U. S. 4515 were used to make up the 24 plants at 20 foot-candles at 60° F., but the data are not represented in the graph.

spontaneum in response to light and temperature were observed. The response of the varieties of the other species of *Saccharum* closely paralleled that of some of the varieties of *Saccharum spontaneum*.

The behavior of plants of all the varieties used in experiments 1 to 3 relative to the presence and absence of growth and the cessation of life at all conditions of light and temperature is shown graphically in figures 2 to 4.

Although temperatures of 60° and 65° F. are near the lower temperature limit for growth of sugarcane, plants grown at these temperatures were, on the whole, more normal than those grown under comparable light intensities at 70° and were in much better health than those grown at the same light intensities at 78° and 86°. The results show clearly that at 78° and 86° and even at 70° a greater light intensity is required for survival, at least during the period covered by these experiments. The contrast in appearance of the plants grown at 65° and those grown at 86° with the same light source (100 foot-candles) in experiment 4 is strikingly illustrated in figure 5. The plants grown

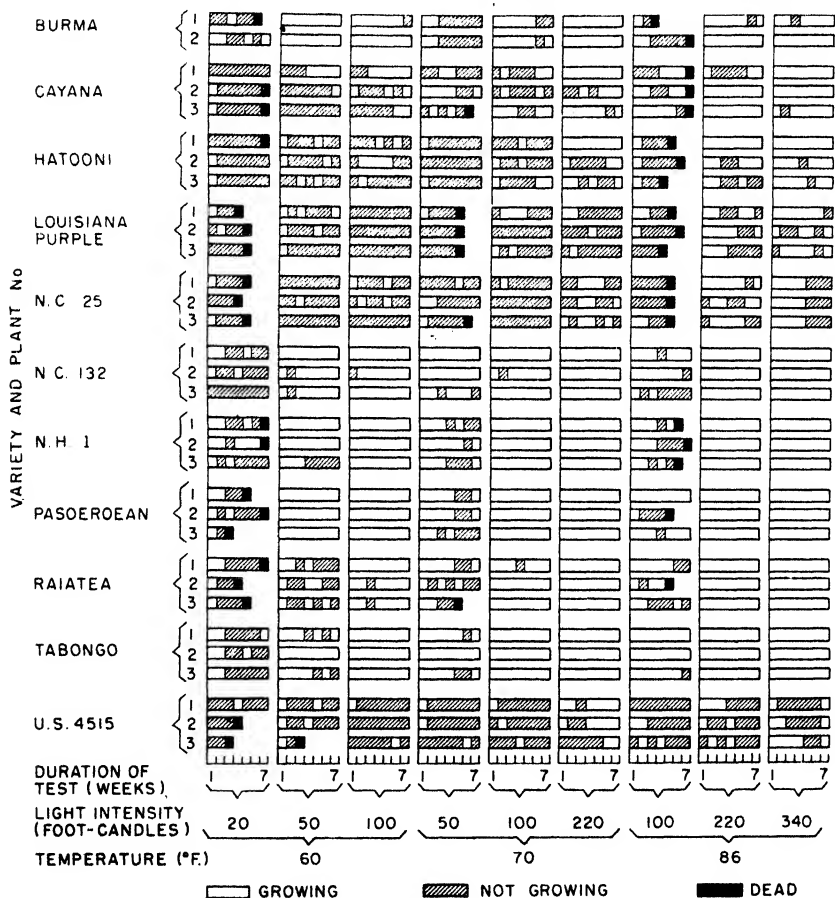


FIGURE 3.—Response of different varieties of sugarcane and erianthus to light intensities at different temperatures in experiment 2.

at 65° showed greater health and less mortality than those grown at 86°. At a light intensity of 100 foot-candles at 60°, growth occurred during more periods (weekly) and more continuously in plants of most varieties in experiments 1 and 2 than at 60° and 65° in experiments 3 and 4 (figs. 2 to 5). At the same intensity at 86°, the plants in experiment 3 were weaker and in many instances death occurred earlier than in plants of the same variety in experiment 2. Associated

with this difference in vital response in the different experiments was a difference in the age of the plants at the beginning of the experiments.

Growth was continuous in most varieties at light intensities of 340 foot-candles at 78° F., 340 and 500 foot-candles at 86°, and 220 foot-candles at 70°.

In addition to the graphically represented distinction between plants exposed to the same light intensity (100 foot-candles) at different temperatures, there was a striking difference in the appearance of the plants. Although growth was not great at 60° and 65° F., the plants, on the whole, exhibited a good green color and appeared nor-

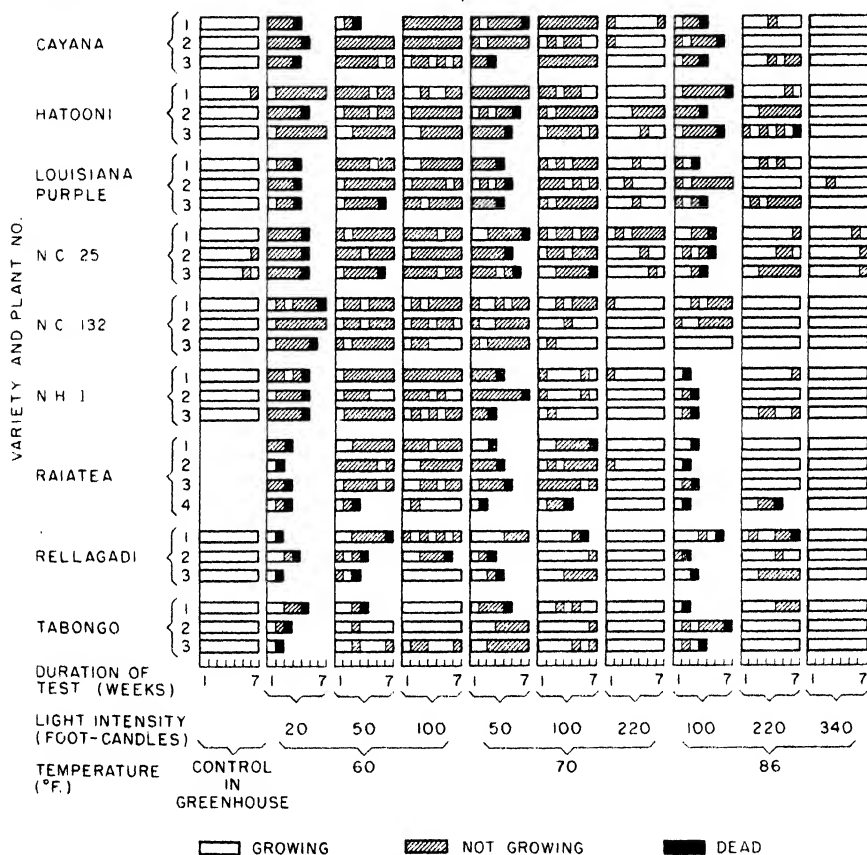


FIGURE 4.—Response of different varieties of sugarcane and erianthus to light intensities at different temperatures in experiment 3.

mal; whereas at 70°, 78°, and 86°, and particularly at the last two temperatures, they showed symptoms of poor health, and in many cases died. The symptoms of poor health consisted of yellowing and dying of the leaves and chlorosis of the spindle,³ particularly from ½ to 2 inches just above the upper leaf sheath. This tissue was often brittle and weak, and in many instances it collapsed and the top of the

³ Unfurled, immature leaves.

plant fell over before death ensued. Similar symptoms were evident at the lower light intensities at 60°, 65°, and 70°.

The average total increases in height per plant of the mother stalks and mother stalks plus suckers in experiments 1 to 4 are given in tables 3 and 4.

As measured by the increase in height of mother stalks alone or of mother stalks plus suckers, most varieties showed an increase in growth with an increase in light intensity at all temperatures. The sums of the total increase in height of individual varieties showed a definite correlation with the light intensities employed at each tem-

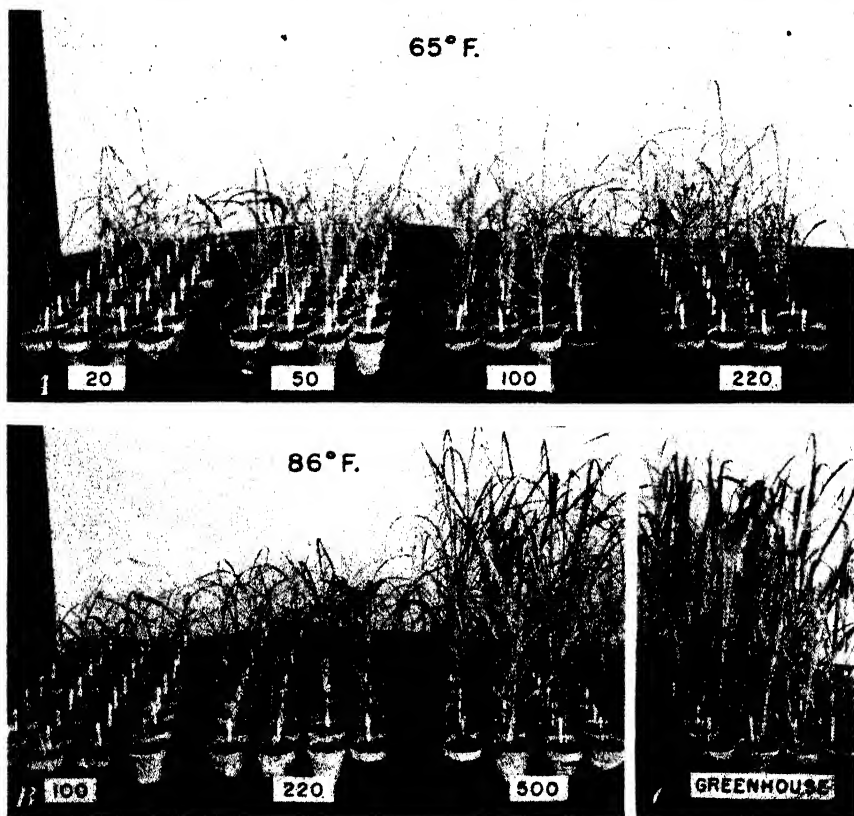


FIGURE 5.—Plants of different varieties of sugarcane grown at 65° F. (A), at 86° (B), and in a greenhouse (C). The numbers below the pots are foot-candles. Note how much larger the plants at 100 foot-candles are at the lower temperature; there was comparatively little difference in the size of those at 220 foot-candles. The temperature generally ranged from 75° to 85° in the greenhouse, where no light-intensity measurements were made. The varieties were Caña Criolla, Co. 281, C. P. 1165, C. P. 29/291, C. P. 31/294, C. P. 31/511, C. P. 34/79, Djatiroto, N. C. 117, and P. O. J. 2725.

perature. It is obvious that the age of the plants affected their rate of growth as well as their survival; that is, rate of growth under the same light intensities was greater in the older plants in experiments 1 and 2 than in the younger plants in experiments 3 and 4.

At a light intensity of 100 foot-candles there was less total growth in all instances (sum of totals of individual varieties) at 78° and at 86°

than at 70° F., and in some instances less than at 60° (table 3). Growth was greater at 70° than at 60°. There was also more injury to the plants at 78° and 86° than at 70°. The results indicate that more light is required for health at 78° and 86° than at 60° and 70°. At a light intensity of 50 foot-candles the total growth was sometimes greater at 60° and sometimes at 70°.

TABLE 4.—Total average increase in height per plant of mother stalks plus suckers and of mother stalks alone of different varieties of sugarcane exposed to various conditions of light and temperature during 4 weeks,¹ in experiment 4

MOTHER STALKS PLUS SUCKERS

Variety	Average initial height of mother stalk	Increase in height with indicated light intensity (foot-candles) at--							Green-house controls
		65° F.				86° F.			
		20	50	100	220	100	220	500	
		<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>
Caña Criolla		8.3	0.3	0.7	0.8	2.0	2.0	2.5	8.7
Co. 281		13.1	.1	.8	.8	1.9	1.6	4.3	17.5
C.P. 1165		12.8	.8	.8	3.7	6.2	1.5	2.3	19.2
C.P. 29/291		14.7	1.5	1.4	1.9	4.1	2.0	4.0	18.4
C.P. 31/294		14.6	.7	.5	1.5	4.3	2.0	5.8	20.3
C.P. 31/511		12.1	1.2	1.8	1.8	3.3	1.8	5.5	20.0
C.P. 34/79		11.9	.3	.7	.8	2.2	1.0	.3	12.2
Djatiroto		13.5	.7	1.0	1.8	6.3	2.5	6.8	30.0
N.C. 117		8.5	.3	.7	.3	.7	2.3	3.7	7.8
P.O.J. 2725		11.8	.7	.8	.3	.8	1.3	1.3	12.5
Total			6.6	9.2	13.7	31.8	18.0	36.5	166.6
									299.4

MOTHER STALKS ALONE

Caña Criolla	8.3	0.2	0.7	0.8	2.0	1.8	2.5	7.2	9.2
Co. 281	13.1	.1	.8	.8	1.9	1.6	4.3	16.3	18.5
C.P. 1165	12.8	.5	.5	1.0	5.8	1.2	2.2	18.8	18.0
C.P. 29/291	14.7	1.3	1.4	1.9	4.1	2.0	4.0	18.3	25.5
C.P. 31/294	14.6	.7	.5	1.5	4.3	2.0	5.8	20.2	23.2
C.P. 31/511	12.1	1.2	1.8	1.8	3.3	1.8	5.5	20.0	19.7
C.P. 34/79	11.9	.3	.7	.8	2.2	1.0	.2	11.8	15.0
Djatiroto	13.5	.3	.8	1.8	5.7	1.5	4.8	23.7	23.7
N.C. 117	8.5	.3	.7	.3	.7	2.3	3.7	7.8	11.8
P.O.J. 2725	11.8	.7	.7	.3	.8	1.3	1.3	12.5	16.7
Total		5.6	8.6	11.0	30.8	16.5	34.3	156.6	181.3

¹ Period covered was June 13 to July 11, 1941. The plants were 64 days old when the experiment was started.

In view of the fact that growth was limited as much at 70° as at 60° F. at a light intensity of 50 foot-candles, it would seem that a light intensity between 20 and 50 foot-candles might be found that would limit growth more at 70° than at 60°.

There was considerable difference in the total growth of varieties grown at the various light intensities at the different temperatures. Some of this difference was related to the size of the plants at the beginning of the experiments. Particularly was this true in experiments 1 and 2. Three factors may have entered actively into this relation. (1) Under uniform conditions in a greenhouse certain varieties grew faster than others, indicating a normal differential rate of growth among varieties. A similar differential rate of growth might be expected among varieties when exposed to different conditions of light and temperature. (2) The difference in height of plants of different varieties at the beginning of an experiment may have

influenced varietal rate of growth. The taller varieties were near the source of light and, therefore, were exposed to higher light intensities than the shorter ones. (3) In some instances, the rate of growth was influenced by the number of suckers present at the beginning of the experiments. Conditions in the greenhouse were much more conducive to suckering than the different conditions of light and temperature used in these experiments. In experiments 3 and 4 the plants of different varieties were more nearly the same size when exposed to the various light intensities and temperatures than those in experiments 1 and 2, yet considerable varietal difference in behavior was evident after exposure. The results of experiments 1 and 2 indicate strongly that some of the varieties behaved differently under the environmental conditions of these experiments. That there is a varietal response is indicated by the results obtained with Louisiana Purple and Rellagadi in experiment 3. Louisiana Purple showed little growth at any of the combinations of light and temperature, whereas Rellagadi showed more growth at all combinations and a great deal more at some, although the plants were smaller than Louisiana Purple at the beginning of the experiment. Similar differences in rate of growth were obtained with these two varieties in experiment 1.

TABLE 5.—Average growth increment per plant of mother stalks plus suckers of different varieties of sugarcane and erianthus exposed for various periods to different conditions of light and temperature in experiment 2

Variety	Temperature	Light intensity	Growth increment after indicated period (weeks)							Total
			1	2	3	4	5	6	7	
Burma	60	Foot-candles	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
		(20)	0.8	0.5	0.8	0	0.3	1.0	0.3	2.7
		(50)	2.3	1.3	2.0	2.5	3.3	2.3	1.8	15.5
	70	(100)	3.5	2.5	2.0	2.3	2.0	1.5	.8	14.6
		(50)	3.5	2.3	0	0	0	0	0	5.8
		(100)	1.8	2.3	1.5	3.3	1.8	0	.3	11.0
	86	(220)	1.8	3.8	.5	2.8	6.5	5.0	3.5	23.9
		(100)	2.5	.5	0	0	0	0	(2)	3.0
		(220)	6.0	2.5	3.5	7.8	3.0	.8	5.5	29.1
	(60)	(340)	5.8	4.8	5.3	12.5	8.3	13.0	12.3	62.0
		(20)	.5	0	0	0	0	0	3.0	.5
		(50)	0	0	0	.3	.2	.2	.5	1.2
Cayana	60	(100)	.2	0	.8	.8	1.3	.5	.5	4.1
		(50)	.2	.7	.3	.5	0	1.0	.7	2.4
		(100)	.7	.7	.2	0	0	.8	.5	2.9
	70	(220)	1.0	.8	1.0	.5	1.2	1.5	1.7	7.7
		(100)	.3	.3	2.0	2.2	1.3	2.0	4.7	8.8
		(220)	1.7	1.3	.5	2.5	1.3	3.0	3.3	13.6
	86	(340)	2.8	2.5	6.8	5.0	6.7	9.5	6.5	39.8
		(20)	.5	0	0	0	0	0	1.0	.5
		(50)	.8	0	.2	0	.3	.2	0	1.5
	(60)	(100)	0	.5	.2	.3	.2	.2	0	1.4
		(50)	.8	0	0	0	0	0	0	.8
		(100)	.5	0	0	.3	0	.2	.5	1.5
Hatooni	70	(220)	.7	.5	.3	.5	.3	1.0	1.3	4.6
		(100)	.8	0	0	1.0	3.0	(2)	.8	.8
		(220)	1.0	.5	.3	.5	4.2	4.0	4.8	15.3
	86	(340)	1.5	2.0	2.2	1.2	1.5	2.2	1.7	12.3
		(20)	.2	.2	0	1.0	(3)			.4
		(50)	1.0	0	.2	0	.2	0	.2	1.6
	(60)	(100)	0	0	0	0	0	0	0	0
		(50)	.3	0	0	0	(3)			.3
		(100)	.3	.3	.3	.2	0	0	0	1.1
	70	(220)	.2	.3	0	.2	0	0	0	.7
		(100)	.5	.2	0	1.0	3.0	(2)		.7
		(220)	1.0	.8	.3	.2	.2	.2	.3	3.0
Louisiana Purple	86	(340)	.5	.5	.8	1.5	.8	.3	.3	4.7

See footnotes at end of table.

TABLE 5.—Average growth increment per plant of mother stalks plus suckers of different varieties of sugarcane and erianthus exposed for various periods to different conditions of light and temperature in experiment 2—Continued

Variety	Temperature	Light intensity	Growth increment after indicated period (weeks)							Total
			1	2	3	4	5	6	7	
	° F.	Foot-candles	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
N. C. 25	60	20	0.3	0	0	1.0	(2)	0	0	0.3
		50	0	0	.2	0	0	0	0	.2
		100	.2	.2	0	0	.3	0	0	.7
	70	50	.5	.2	0	0	.2	1.0	0	.9
		100	0	.2	0	0	0	0	0	.2
		220	.2	0	.5	.5	.2	.2	.2	1.8
	86	100	.2	.2	0	0	(2)	0	0	.4
		220	.3	1.7	.5	1.0	.5	1.3	2.3	7.6
		340	.7	2.3	1.7	1.3	0	0	0	6.0
	60	20	.7	.2	0	.2	.2	0	0	1.3
		50	.8	.3	1.0	1.3	1.0	1.5	1.5	7.4
		100	.5	.8	1.8	2.0	2.0	2.5	1.7	11.3
N. C. 132	70	50	2.2	1.0	1.3	1.8	2.3	1.8	.5	10.9
		100	.5	.3	.7	1.5	3.5	2.0	2.5	11.0
		220	1.3	2.2	2.7	2.8	3.0	3.2	3.5	18.7
	86	100	3.0	.7	1.2	.5	2.2	2.8	.7	11.1
		220	3.0	3.0	4.0	7.2	5.0	3.7	5.0	30.9
		340	2.3	3.7	5.3	6.2	5.8	6.3	6.0	35.6
	60	20	2.0	.3	.2	.2	.3	.2	3.0	3.2
		50	1.2	1.0	1.0	.8	1.0	.3	.5	5.8
		100	1.3	.8	2.0	2.3	2.0	2.0	1.5	11.9
	70	50	4.2	1.3	.8	1.0	.5	0	.5	8.3
		100	3.3	2.5	1.8	1.8	1.8	2.3	3.3	16.8
		220	4.0	2.7	2.7	3.8	3.8	3.7	4.5	25.2
N. H. 1	86	100	5.7	.7	.5	.2	.2	(2)	(2)	7.3
		220	6.0	6.0	5.8	8.0	6.7	4.2	5.3	42.0
		340	5.3	7.0	5.8	7.8	7.2	6.7	6.7	46.5
	60	20	1.3	.2	1.2	0	3.0	0	(2)	1.7
		50	1.2	.7	1.0	1.3	1.2	.8	.8	7.0
		100	1.3	1.2	1.5	1.7	2.3	3.0	2.8	13.8
	70	50	3.5	3.5	2.0	.8	0	0	1.2	11.0
		100	2.8	3.7	3.7	3.3	2.7	2.2	2.3	20.7
		220	4.0	6.7	6.0	6.0	5.5	5.8	8.5	42.5
	86	100	5.5	1.5	.8	1.8	2.5	4.5	.8	17.4
		220	8.3	11.8	11.3	10.8	13.2	9.5	7.0	71.9
		340	7.2	14.0	13.2	13.2	17.2	10.8	13.7	89.3
Pasceroean	60	20	1.0	.2	0	1.0	3.0	0	(2)	1.2
		50	.5	.2	0	.5	.2	.2	0	1.6
		100	.7	.5	.2	.8	.7	.5	.5	3.9
	70	50	2.5	.5	.3	.3	1.2	0	.2	4.0
		100	1.5	.5	.8	.8	1.8	2.7	3.5	11.6
		220	1.2	1.3	1.8	2.7	4.5	4.7	5.2	21.4
	86	100	1.7	.3	.5	.3	1.2	.2	0	3.2
		220	3.8	3.2	4.8	5.8	5.2	3.2	4.8	30.8
		340	4.0	6.3	9.5	10.0	7.8	7.8	7.7	53.1
	60	20	2.0	.8	0	0	.2	0	.2	3.2
		50	2.3	1.8	1.0	.7	.5	1.5	1.7	9.5
		100	2.8	1.7	2.0	1.3	2.8	2.3	2.5	15.4
Tabongo	70	50	5.0	3.3	4.2	1.8	1.5	.8	1.2	17.8
		100	5.5	5.3	2.7	3.0	4.2	4.2	3.2	28.1
		220	4.8	7.2	6.3	4.5	7.8	5.3	7.5	43.4
	86	100	6.2	5.5	3.8	4.5	5.2	3.5	1.7	30.4
		220	10.0	11.8	11.3	6.8	10.7	12.7	15.2	78.5
		340	12.0	18.5	12.5	16.3	21.5	14.5	14.2	109.5
	60	20	0	0	1.0	3.2	0	0	0	.2
		50	2.0	0	1.0	.2	.2	0	0	2.4
		100	.2	0	0	0	0	.5	0	.7
	70	50	1.2	0	0	0	0	.2	0	1.4
		100	.2	.2	0	.2	0	0	0	.6
		220	1.7	.3	0	1.0	.8	.8	.7	5.3
U.S. 4515	86	100	.2	.5	0	.3	0	0	0	1.0
		220	.3	.3	.2	.3	0	0	0	1.1
		340	1.7	.5	.3	.3	0	0	.8	3.6

¹ 1 plant dead.² All plants dead.³ 2 plants dead.⁴ Growth due to suckers; mother stalks dead.

The rate and change of rate of growth of the varieties in experiment 2 (table 5) serve to differentiate clearly not only the effects of temperature and light intensity but also varietal behavior. Very little

growth occurred in Hatooni, and even less in N.C. 25, Louisiana Purple, and U.S. 4515, as compared with other varieties at most of the conditions of light and temperature. In the varieties that showed considerable growth there was a tendency for the rate of growth at low light intensity at each of the temperatures to decline with the lapse of time. This decline was sometimes more marked at the higher temperatures, although the low light intensity was higher at the higher temperatures than at the low. On the other hand, at the higher intensities at all the temperatures, and particularly at the higher temperatures, the initial rate of growth was more often maintained and in some instances increased during the succeeding periods of exposure. At least part of this more favorable response at the higher temperatures can be attributed to the higher light intensities employed as compared with those at the lower temperatures. At low intensities of light the rate of growth tended to be higher during the first week than during the later weeks of exposure. This tendency was apparent at many of the intensities in varieties that showed little growth at any of the conditions of light and temperature. It is possible that the preceding conditions of growth in the greenhouse may have had some effect upon this initial period under experimental conditions.

In experiment 5 (table 6) one plant each of two varieties was exposed to light intensities of 200 and 1,750 foot-candles at 86° F. for 4 weeks. It will be seen that the higher intensity (1,750 foot-candles) was much more favorable for growth than the light intensities used in the other experiments (1 to 4). None of the experiments, of course, was designed to determine the optimum light intensity; nor is it definitely known whether the optimum light for the growth of sugarcane is ever exceeded in nature. Clements (3) has shown, however, that under natural conditions light intensity may become too low for maximum growth.

TABLE 6.—Growth increments in plants (mother stalks plus suckers) of 2 varieties of sugarcane exposed for 4 weeks¹ to 2 light intensities at 86° F. and a relative humidity of about 75 percent, experiment 5

Variety	Light intensity	Initial height of mother stalk	Growth increment after indicated period (weeks)				Total increase in length	Dry weight
			1	2	3	4		
	<i>Foot-candles</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Gm.</i>
Burma	200	10	4.5	0.5	6.0	2.5	13.5	1.2
	1,750	12	16.5	51.5	76.5	56.5	201.0	20.3
Cayana	200	21	5.0	3.0	10.5	5.5	24.0	4.7
	1,750	19	18.5	25.0	23.5	19.0	86.0	40.2

¹ The period covered was Jan. 10 to Feb. 7, 1941. The plants were 94 days old at the beginning of the experiment.

Tables 7 and 8 show the average dry weights of plants at the different conditions of light and temperature obtained in experiments 1 to 4. In addition to these data, dry weights of control plants grown in the greenhouse in connection with experiments 3 and 4 are shown in tables 7 and 8. The initial weights of plants representative of those exposed to the various conditions of light and temperature in experiment 4 are given in table 8.

TABLE 7.—Average dry weight of plants of different varieties of sugarcane and erianthus exposed for 7 weeks to various conditions of light and temperature in 3 experiments

Experiment and variety	Dry weight with indicated light intensity (foot-candles) at—									Green-house controls
	60° F.			70° F.			78° or 86° F. ¹			
	20	50	100	50	100	220	100	220	340	
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
Experiment 1:										
Burma	12.4	14.1	11.7	15.6	12.4	21.3	15.3	16.9	20.3	
Cayana	6.6	6.7	10.0	6.0	10.6	12.2	8.1	11.3	26.0	
Gehra Bon.	5.4	7.5	8.4	4.8	4.4	7.4	4.9	7.2	9.1	
Hatooni	7.2	8.3	11.7	5.8	9.3	10.8	9.0	10.2	11.7	
Louisiana Purple	7.0	7.0	7.7	7.0	7.9	8.0	5.0	9.9	12.0	
N. C. 132	5.8	7.5	6.0	5.8	6.2	8.0	5.3	9.2	15.1	
N. H. 1	8.9	8.1	13.8	10.8	14.9	15.1	11.5	14.5	23.2	
Paseroean	8.6	12.0	14.2	6.9	7.4	9.3	10.3	14.9	17.8	
Rellagadi	12.4	14.2	15.4	9.8	12.0	17.4	11.4	15.6	19.3	
Tabongo	10.4	13.7	14.1	10.9	13.3	17.8	12.2	15.5	21.1	
28 N. G. 7	5.5	8.1	8.3	5.2	5.1	10.5	5.7	9.2	16.8	
Total	90.2	107.2	121.3	88.6	103.5	137.8	98.7	134.4	192.4	
Experiment 2:										
Burma	2.3	3.8	2.2	2.1	2.2	2.5	1.9	4.0	5.6	
Cayana	3.4	4.0	3.9	4.0	4.0	5.1	3.5	4.3	9.3	
Hatooni	2.6	3.1	3.6	3.0	3.9	4.8	2.4	4.4	8.4	
Louisiana Purple	3.3	3.2	2.4	2.3	3.0	3.0	1.7	2.8	4.9	
N. C. 25	3.9	3.8	4.1	4.4	2.4	3.2	2.1	3.7	7.6	
N. C. 132	4.7	4.9	4.7	4.3	3.2	4.2	3.1	4.4	7.0	
N. H. 1	6.3	6.8	7.9	6.7	6.7	7.7	5.0	9.9	11.8	
Paseroean	4.2	4.7	4.6	3.9	4.3	6.1	4.2	6.3	7.7	
Raiaata	3.8	4.3	4.3	4.1	5.1	5.7	3.3	7.8	11.2	
Tabongo	4.6	6.1	6.5	5.6	5.4	6.7	5.9	5.7	9.6	
U. S. 4515	3.0	1.9	2.6	2.8	2.3	2.6	2.5	2.7	2.4	
Total	42.1	46.6	46.8	43.2	42.5	51.6	35.6	56.0	85.5	
Experiment 3:										
Cayana	2.5	3.5	.1	3.1	3.5	3.2	2.1	3.8	6.0	22.3
Hatooni	4.8	4.7	5.5	3.6	4.3	6.7	3.7	5.5	11.1	34.3
Louisiana Purple	1.9	1.9	2.1	1.9	2.3	3.9	2.9	5.2	6.8	28.5
N. C. 25	2.3	2.3	1.7	2.2	2.4	4.0	2.6	4.4	5.6	21.0
N. C. 132	1.2	1.6	1.5	1.3	2.9	2.4	2.4	2.8	3.5	17.0
N. H. 1	2.5	3.0	1.9	1.7	2.8	5.7	2.9	3.2	6.4	28.1
Raiaata	2.8	2.5	2.7	2.5	2.2	3.5	1.9	3.6	5.2	
Rellagadi	1.5	2.0	1.5	1.9	1.4	2.6	1.3	1.4	2.9	31.0
Tabongo	1.2	2.1	1.9	1.9	1.9	3.0	1.4	2.5	3.6	20.2
Total	20.7	23.6	21.9	20.1	23.7	35.0	21.2	32.4	51.1	292.4

¹ 78° in experiment 1 and 86° in experiments 2 and 3.² There were no plants of Louisiana Purple and N. C. 132 at 20 foot-candles at 60° F. in experiment 1. Because the dry-weight values of these varieties were similar to those at 20 foot-candles at 60° and at 50 foot-candles at 70° (experiments 2 and 3), the dry-weight values of these varieties (Louisiana Purple, 7 gm.; N. C. 132, 5.8 gm.) in this experiment were used in computing the total dry weight at 20 foot-candles at 60°.

In experiment 1 the relation between light intensity at each temperature is clearly seen in connection with most varieties, the dry weights increasing with the intensity of the light. This relation holds at 86° F. in experiment 2 (table 7). At 70° in experiment 2 the tendency for the dry weights to increase with the light intensities is offset by exceptions. However, in most instances the dry weights are higher at the highest light intensity than at the two lower intensities. At 60° (table 7, experiment 2) the dry weights at the two highest intensities are in some instances slightly greater than at the lowest, but the difference is not marked. In experiment 3 (table 7) there appears to be no relation between dry weights and light intensity at 60°; at 70° there appears to be a relation in some varieties, and at 86° the correlation is clear. In experiment 4 (table 8) the three lowest light intensities at 65° seem to have little or no effect on dry weight. The highest light intensity at this temperature seems

to have caused an increase in dry weight in some varieties. At 86° the dry weights increased with the light intensity. In general, although not as conclusively as the growth increments (increase in length of stalk), dry weight indicates a dependence on light intensity for its production. At the higher temperatures this relation is clear. At the lower temperatures, which themselves become a limiting factor, the effect of light intensity is often not evident. However, the higher light intensities maintain a much better condition of health in the plants at each of the temperatures than the lower light intensities. The dry-weight data in these experiments indicate that the low light intensities (50 and 100 foot-candles) at 78°, 86°, and 70° are no more favorable, and in some instances apparently less favorable, to the production of dry matter than these same light intensities at 60°, although these higher temperatures are normally much more favorable to growth than the lower ones.

TABLE 8.—Average initial dry weights and average dry weights of plants of different varieties of sugarcane exposed for 4 weeks to various conditions of light and temperature; experiment 4

Variety	Initial dry weight	Dry weight with indicated light intensity (foot-candles) at—							Green house controls
		65° F.				86° F.			
		20	50	100	220	100	220	500	
	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Caña Criolla	1.5	1.7	1.6	1.6	1.9	1.4	1.8	3.3	10.6
Co. 281	2.5	2.2	2.5	1.9	2.3	2.5	2.2	5.7	11.3
C. P. 1165	2.7	3.0	2.5	3.6	4.1	2.9	3.6	6.3	13.5
C. P. 29/291	2.3	2.6	2.2	2.8	3.3	2.5	3.3	5.7	15.0
C. P. 31/294	2.2	1.8	2.4	2.1	3.2	2.4	2.7	5.6	13.3
C. P. 31/511	3.2	2.7	3.0	2.5	3.8	2.8	3.8	5.8	14.4
C. P. 34/79	2.4	2.3	2.9	2.7	3.9	2.6	3.4	7.0	12.2
Djatirot	2.2	1.6	1.7	1.9	1.9	1.8	2.0	4.8	11.0
N. C. 117	1.2	1.1	1.6	1.1	1.6	1.3	1.7	2.9	9.2
P. O. J. 2725	2.5	2.2	2.8	2.5	2.8	2.6	2.6	7.4	13.6
Total	22.7	21.2	23.2	22.7	28.8	22.8	27.1	54.5	124.1

In the foregoing experiments the growth response of the several varieties to the light-temperature combinations employed seemed to bear no relation to the latitudes in which the varieties originated, but this may be attributable to limitations of the environmental factors artificially set up, especially the deficiency of light in most experiments. Field observations strongly hint that at relatively low temperatures the northern varieties respond to the stimulus of adequate light (daylight at Arlington Experiment Farm, Arlington, Va.) and grow faster than equatorial varieties. In the experiments under consideration, however, Louisiana Purple, coming from latitude 6° S., behaved similarly in its growth response to U. S. 4515, originating at latitude 40° N. Both varieties showed little growth under any of the environmental conditions. The noble canes (Louisiana Purple, Caña Criolla, N.C. 25, and N.C. 117), varying in their origin from latitude 6° S. to 11° and 21° N., showed relatively little growth at any of the combinations of light and temperature.

DISCUSSION

The minimum light requirement for health and growth of sugarcane in the experiments here reported may have been conditioned only by

the compensation point between the anabolism of photosynthesis and the catabolism of respiration. If the destruction was greater than the synthesis of carbohydrates, the plants suffered starvation as a result of a shortage of this type of food. It is possible, however, that the plants may have suffered injury as a result of direct or indirect effects of limiting light intensities on such factors as other phases of nutrition, absorption of solutes, and transpiration, even when the amount of carbohydrates in the plants exceeded that required in respiration.

The results of studies by McLean (5), Yap (7), and Gooding (4) indicate the possibility that light intensity may become too great during midday in the Philippine Islands and in Barbados, British West Indies, for the maximum rate of photosynthesis in sugarcane. The results of McLean obtained in the Philippines showed a maximum rate of photosynthesis during the morning between 7:30 and 9:30 and in the afternoon between 3:30 and 5:30 (5). Yap, working in the Philippines, found the maximum rate of photosynthesis between 8 and 10 a. m., a depression between 10 a. m. and 2 p. m., and an increase between 2 and 4 p. m. It is possible, and perhaps probable, that the midday depression of the rate of photosynthesis was the result of indirect effects such as those produced by high temperatures and disturbed moisture relations. Yap noted that during the midday period there was a rolling of the tips of the leaves. He also reported greater photosynthetic activity in young leaves, and a decreasing rate with age.

Singh and Lal (6), working in India with severed leaves of the Reori variety of sugarcane at a temperature of 30° C. (86° F.) and with a normal carbon dioxide content of the air, found an increase in the rate of photosynthesis with an increase in light intensity from 90 to 1,875 foot-candles, the highest intensity used. At an atmospheric concentration of carbon dioxide and a light intensity of 1,875 foot-candles, there was an increase in the rate of photosynthesis with a rise in temperature from 23° C. (73.4° F.) to 30° C. (86° F.) and an initial increase in rate with a rise from 30° C. to 34° C. (93.2° F.), followed by a decline in rate at 34°.

The different responses of the varieties and species of sugarcane to minimum light requirements imply considerable practical significance, especially if there should prove to be different responses at higher light intensities to various temperatures. Such varietal differences would provide the basis for considerable adaptation to a particular climate through breeding and selection.

SUMMARY

The data here presented deal with the effects of different but relatively low light intensities, at temperatures of 60°, 65°, 70°, 78°, and 86° F., on the health, growth, and survival of plants of 21 varieties of sugarcane and 3 varieties of *erianthus*.

There was a varietal response to the conditions of light and temperature.

In general, the health of the plants improved and growth increased with an increase in light intensity at each of the temperatures.

For each temperature there was a minimum light intensity for survival, health, and growth; the higher the temperature the higher

that minimum became. With an increase in light intensity up to approximately 220 foot-candles at 65°, and to 1,750 foot-candles at 86°, there was increased growth. At 60° and a light intensity of approximately 100 foot-candles the anabolic and catabolic processes approached a balance and the plants remained in fairly good health for 7 weeks, whereas under this same light intensity at 78° and 86°, particularly at 86°, the life of the plants was impaired and death was common.

These data suggest that, for every temperature within the normal range of temperatures occurring naturally, a definite quota of light is required for maximum growth.

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THE ARGENTINE CURLY TOP OF SUGAR BEET¹

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INTRODUCTION

In 1925 Fawcett (8)² described a disease, called encrespamiento, of sugar beets (*Beta vulgaris* L.) in the Province of Tucumán, Argentina. He reported that it produced symptoms indistinguishable from those caused by curly top of sugar beets in the western part of the United States. However, Fawcett (9, 10), Henderson (16), and Severin and Henderson (20) were unable to find *Eutettix tenellus* (Bak.), the vector of the virus of North American curly top, in Argentina. Fawcett showed that the vector of the Argentine curly top virus is a leafhopper that at different times has been identified as *Aceratogallia sanguinolenta* (Prov.) (8) and (*Agallia*) *Agalliana sticticollis* (Stål.) (10). This leafhopper has been described more recently by Oman (18) as a distinct species, *Agalliana ensigera*.

Curly top is extremely destructive to sugar beets and other crops in the western part of the United States. Until the introduction of curly-top-resistant varieties almost complete abandonment of the sugar-beet industry in several of the Western States was threatened because of this disease.

Because of the similarity of the Argentine disease to North American curly top and its potential economic significance if introduced into the sugar-beet-producing areas of other parts of the world, the disease has been of considerable interest to the sugar-beet industry of the United States. At different times efforts have been made to obtain additional information regarding it through direct studies of diseased material. On two occasions, in 1927 and again in 1937, diseased plants were obtained from Tucumán and by special arrangement with the plant-quarantine authorities were brought to Washington, D. C., for study and comparison with North American curly top in quarantine greenhouses. In the season of 1940-41 further studies of the disease were made by the senior author at the Estación Experimental Agrícola de Tucumán in Argentina. The results of all these studies are presented in this paper.

¹ Received for publication May 31, 1944.

² Italic numbers in parentheses refer to Literature Cited, p. 47.

COMPARISON OF SYMPTOMS AND TRANSMISSIBILITY OF ARGENTINE AND NORTH AMERICAN CURLY TOP³

In 1927 plants⁴ of sugar beet and chard (*Beta vulgaris* var. *cicla* L.) affected with curly top were brought from Tucumán, Argentina, to Washington, D. C., and placed in a quarantine greenhouse at the Arlington Experiment Farm, Arlington, Va., for study in comparison with North American curly top. New growth from both kinds of plants showed leaf curling, veinlet clearing, and vein swelling indistinguishable from the symptoms that characterize the curly top disease in the United States. Drops of exudate were observed on badly diseased petioles of both the sugar-beet and the chard plants similar to those found on petioles of sugar-beet plants affected with North American curly top. Transverse sections of badly affected petioles showed vascular necrosis. Symptoms of Argentine curly top, in comparison with those of North American curly top, are illustrated in figures 1 and 2.

Tests were made in which nonviruliferous beet leafhoppers (*Eutettix tenellus*) were caged for different periods on the diseased sugar-beet and chard plants received from Argentina and were later transferred to small sugar-beet plants. As shown in table 1, where the results of these tests are summarized, no infection was obtained, although a high percentage of the check plants on which leafhoppers from plants affected with North American curly top were caged became infected. Plants from one of these tests, illustrating the results obtained with leafhoppers that had fed on diseased Argentine and North American plants, respectively, are shown in figure 3.

A transmission test with an aphid thought to be *Myzus persicae* (Sulz.) also gave negative results.

In 1937 a second lot of diseased plants, consisting of the sugar-beet variety U. S. 12 and the mangel-wurzel (*Beta vulgaris*) varieties Yellow Tankard and Red Mammoth, was obtained from Argentina through the courtesy of G. L. Fawcett, for further experimental work in the quarantine greenhouse at Arlington, Va. These plants showed all the symptoms typical of curly top in the United States, such as leaf curling, veinlet clearing, vein swelling, and production of exudate on diseased petioles, that had been observed on plants of the 1927 importation.

TABLE 1.—Results of attempts to transmit North American curly top and Argentine curly top to sugar beet by means of *Eutettix tenellus* in quarantine greenhouse, Arlington, Va., 1927 and 1937-39

Year	Disease affecting plants on which the beet leafhoppers fed	Plants inoculated by means of beet leafhoppers	Plants infected	
		Number	Number	Percent
1927.....	North American curly top.....	18	15	83.3
	Argentine curly top.....	34	0	0
1937-39.....	North American curly top.....	80	46	57.5
	Argentine curly top.....	127	0	0

³ Experimental work performed by Eubanks Carsner, G. H. Coons, and E. W. Brandes.

⁴ These plants were obtained from G. L. Fawcett, of the Estación Experimental Agrícola de Tucumán, and were brought to Washington through the courtesy of C. F. Henderson, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

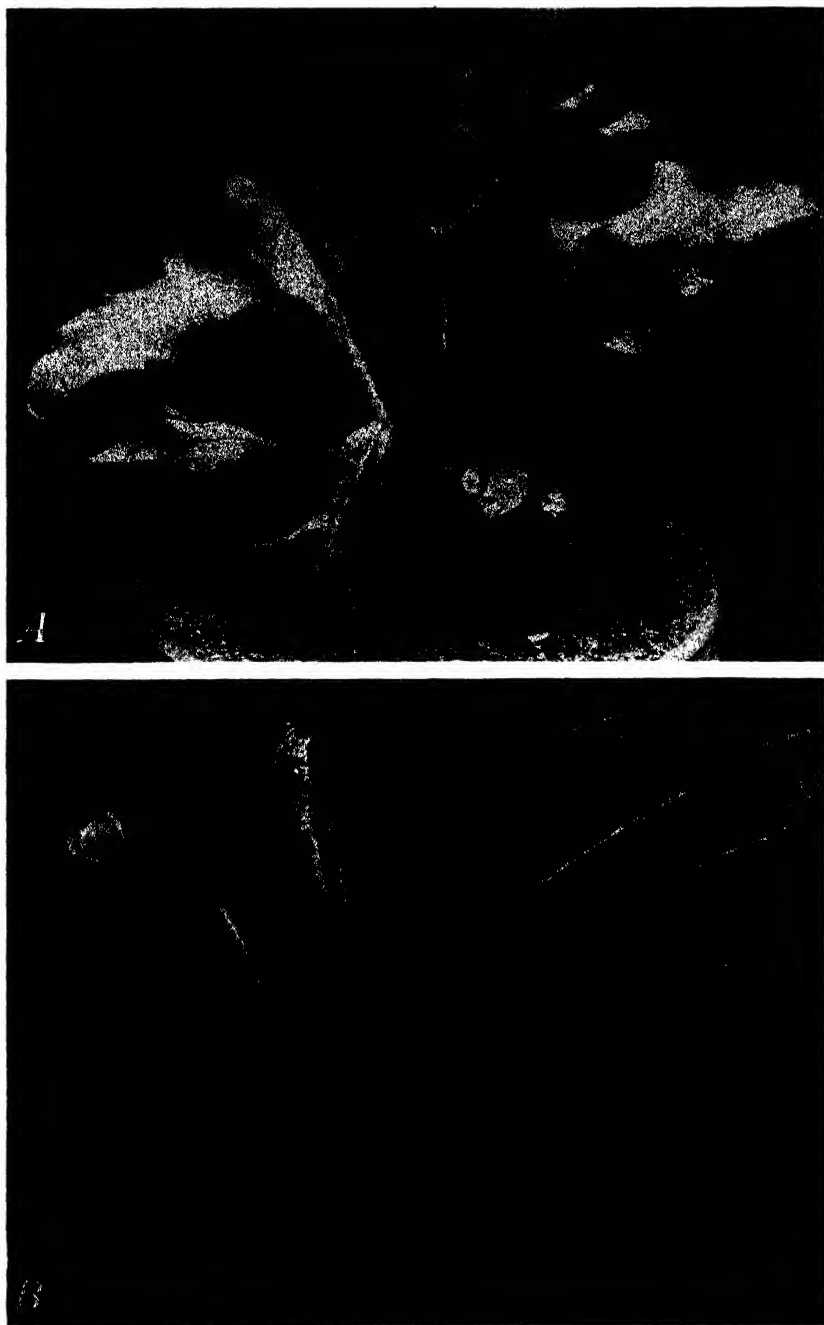


FIGURE 1.—A, Sugar-beet plant affected with Argentine curly top, showing typical leaf curling, vein swelling, and roughened under side of leaves; photographed at Arlington, Va., 1927. B, Sugar-beet plant affected with North American curly top, showing typical symptoms of this disease; photographed at Riverside, Calif., 1943.

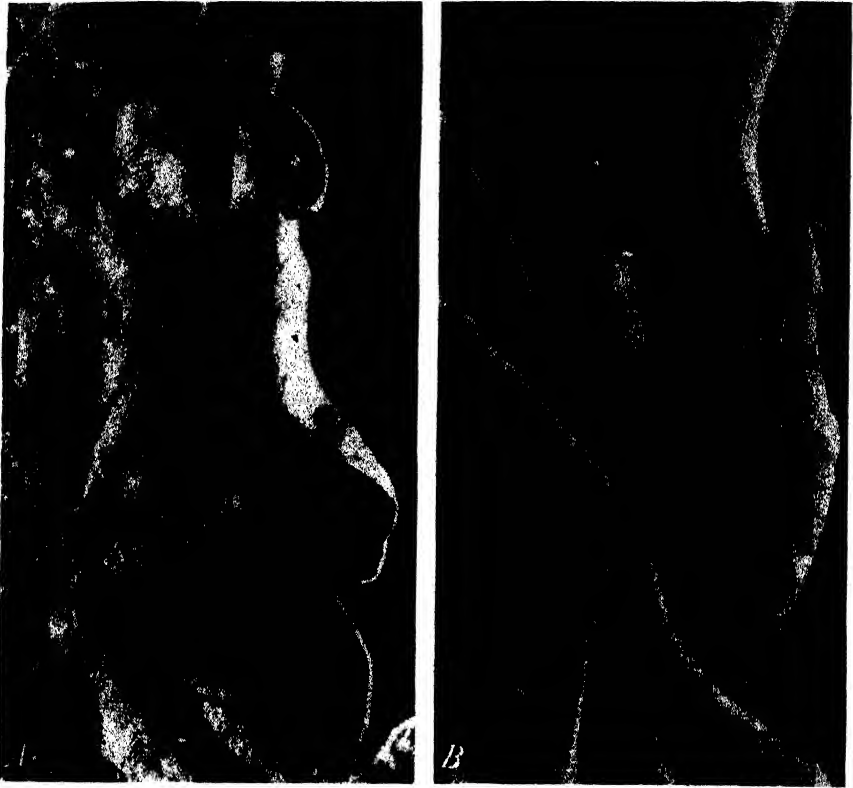


FIGURE 2.—Vein protuberances on under side of leaves of sugar-beet plants affected with (A) Argentine curly top and (B) North American curly top; number and distribution of papillae are probably not distinctive characteristics. Photographed at Arlington, Va., 1927; \times about 5.



FIGURE 3.—A, Sugar-beet plants to which beet leafhoppers (*Eutettix tenellus*) were transferred from diseased Argentine plants; B, sugar-beet plants to which beet leafhoppers were transferred from diseased North American plants. Only North American curly top was transmitted by the beet leafhoppers. Photographed at Arlington, Va., November 29, 1927.

This material was used in further attempts to obtain transmission of the Argentine curly top virus by means of the beet leafhopper. Parallel tests were conducted in which sugar-beet plants affected with North American curly top were the virus source. In an extensive series of tests, nonviruliferous beet leafhoppers were allowed to feed on diseased plants and later were transferred to small sugar-beet plants. The results of these tests, also summarized in table 1, are similar to those obtained earlier in that no infection by the Argentine virus was obtained, whereas the percentage of infection was relatively high on control plants inoculated by means of beet leafhoppers that had fed on plants infected with the North American virus.

Attempts were made also to transmit the Argentine virus by juice inoculation. In these tests juice was extracted from diseased beet leaves, diluted 1 part juice to 1 part water, and introduced into beet plants with about six true leaves by means of numerous punctures into the crowns. Small insect pins were used for making the punctures through drops of inoculum placed on the crowns. The 76 plants inoculated showed no infection. In a second type of test, inoculum was rubbed over the surface of the leaves on which carborundum had been sprinkled previously. The 12 plants so treated showed no infection.

In 1935 and again in 1937 dried leaves were obtained from diseased beets from Tucumán. These were ground to a powder and soaked overnight in distilled water. The liquid was extracted and added to an equal volume of 95-percent alcohol. The resulting precipitate was dried, mixed with 5-percent sugar solution to which 0.5 percent of sodium citrate was added, and fed to nonviruliferous beet leafhoppers, which were then caged singly on seedling sugar beets. Extensive tests were made from each of the two lots of leaves but no infection was obtained. With this technique the virus of North American curly top has been recovered from beet leaves after they had remained dry for 3 years (1).

Dried leafhoppers (*Agalliana ensigera*), sent by G. L. Fawcett from Tucumán, were used in three inoculation tests in November and December 1937. One lot of the leafhoppers had been caged on a diseased beet for more than 3 months, and another lot was collected from a field of completely diseased beets just before mailing on October 29, 1937. On November 15 and 30 and December 9, dried leafhoppers were ground in a mortar and an alcoholic precipitate was obtained. The precipitate was dried and suspended in 5-percent sugar solution. Nonviruliferous beet leafhoppers were allowed to feed on the suspension and then were caged singly on healthy young sugar beets, 88 plants being thus inoculated. None became diseased. Comparable tests by Bennett, not previously reported, with the North American curly top virus and dried beet leafhoppers have shown that the virus could be recovered from the dried insects after 3 months.

In January 1938, four sugar-beet plants, numbered 1 to 4 and known to be infected by the Argentine virus, were inoculated with North American curly top. Plant 1 died about the middle of February, but the remaining three plants were in good condition on July 10 and none showed any increase in severity of symptoms. On that date nonviruliferous beet leafhoppers were caged on these plants and later caged singly on seedling beets. Eight plants inoculated from plant 2 re-

mained healthy, but five of seven and four of six plants inoculated by means of leafhoppers from plants 3 and 4, respectively, became infected. These results show that the strain of North American virus used was superimposed on the Argentine curly top virus in at least two of the four plants inoculated.

The transmission tests conducted with the Argentine virus appear to have been extensive enough to permit the conclusion that this virus is not transmissible by *Eutettix tenellus*. The failures to obtain transmission are all the more striking because of the high percentages of infection obtained in parallel tests in which the beet leafhopper had access to virus of North American curly top. Hence, although the viruses from the two geographical areas produce symptoms that, so far as observed, are indistinguishable on sugar beets, each is associated in its geographical range with a specific leafhopper vector, and the vector of North American curly top virus is unable to transmit the Argentine virus. Whether *Agalliana ensigera* is unable to transmit North American curly top virus is not known.

THE DISEASE IN ARGENTINA ⁵

GEOGRAPHIC DISTRIBUTION

In 1923 Bonequet (4) described curly top on small beets at San Isidro, a suburb of Buenos Aires, as occurring commonly during the season of 1916-17. He reported also that the disease was very destructive in beet plots in Colonia Alvear in western Argentina.

Fawcett (8) in 1925 gave a detailed description of encrespamiento, or curly top, on sugar beet in the Province of Tucumán.

In the season of 1926-27 Henderson (16) found beets with typical foliage symptoms of curly top in the Provinces of Tucumán, San Juan, and Mendoza and in the Territory of La Pampa, but failed to find the disease in the Territory of Río Negro. From an extensive search in the field and the study of insect collections of museums and of entomologists, no evidence was obtained of the occurrence in Argentina of *Eutettix tenellus*, the vector of curly top virus in North America.

In 1939 A. M. Offermann collected *Agalliana ensigera* on sugar beets near Conesa, Río Negro, and found that when these insects were placed on sugar beets at the Government experimental station at José C. Paz the beets developed curly top.⁶

A colony of these insects, kindly supplied by A. M. Offermann, was taken to Tucumán in September 1940 and tested on sugar-beet seedlings of the varieties R. & G. Old Type, U. S.⁷ 11, U. S. 12, and S. L.⁸ 68. Plants of these four varieties proved to be susceptible to infection and developed symptoms indistinguishable from those produced on the same varieties by *Agalliana ensigera* collected at Tucumán. These results indicate clearly that the virus is present in the lower Río Negro Valley. However, a careful search for the disease in

⁴ Experimental work was performed by C. W. Bennett. Appreciation is expressed to Dr. Wm. E. Cross, Director of the Estación Experimental Agrícola de Tucumán, for facilities provided during the course of this work. Indebtedness to Ing. G. L. Fawcett, pathologist, Mr. Kenneth J. Hayward, entomologist, Ing. Enrique F. Schultz, subdirector and horticulturist, Ing. Isaac Manoff, chemist, and Ing. G. A. Kreibohm de la Vega, cotton specialist, is acknowledged for much valuable assistance while the work was in progress.

⁵ Unpublished information communicated to C. W. Bennett by Ing. Alfredo M. Offermann, pathologist, Ministerio de Agricultura, Buenos Aires, Argentina.

⁷ Varieties released for commercial use by the Bureau of Plant Industry, Soils, and Agricultural Engineering are designated as "U. S." varieties. "S. L." refers to varieties maintained at the U. S. Sugar Plant Field Laboratory at Salt Lake City, Utah.

beet plantings, from January 12 to February 3, 1941, in the valley of the lower Río Negro, revealed no infected plants, although the vector (*A. ensigera*) was found in small numbers.

There is evidence, therefore, that the disease is rather widely distributed over the larger part of Argentina north of the Río Negro. Henderson (16) failed to find the disease in 1928 at La Sierra, Uruguay, and inspections of extensive acreages by the senior author in this same area in February 1941 revealed no evidence of curly top.

Sugar beets are not grown commercially in the Province of Tucumán. In and near the city of Tucumán the disease was found only in the vicinity of the Estación Experimental Agrícola de Tucumán. A plot of mangel-wurzels planted in April 1940 was inoculated by G. L. Fawcett by means of viruliferous leafhoppers from stock colonies. This plot provided an excellent source of leafhoppers and virus for infection of plants in adjacent areas. Spread, however, was not extensive, and no diseased plants were found at a distance greater than one-half mile from the plot.

Search was made for curly top in a number of localities in the Province of Tucumán. Native vegetation and cultivated plants were examined at Tafi Viejo, Lules, Ingenio Concepción, Ingenio Bella Vista, and a number of places in the immediate vicinity of the city of Tucumán. In each of these places it was found that beets and other plants known to be susceptible to curly top were grown only to a limited extent. One short row of red garden beets (*Beta vulgaris*) was found north of Tafi Viejo, and another planting of about 25 plants was found south of Lules; but no curly top was observed at either place. The scarcity of types of plants on which curly top could be recognized made it difficult to obtain information regarding the distribution of the virus, and it was not possible to determine the extent to which virus is able to spread from native or escaped plants to cultivated plants under natural conditions.

HOST RANGE

PLANTS INFECTED NATURALLY

In Argentina curly top has been reported on sugar beet (8) and tomato (*Lycopersicon esculentum* Mill.) (11). As will be seen later, however, there is some doubt as to the identity of the tomato disease considered to be curly top.

From October 11, 1940, to February 10, 1941, curly top was found under field conditions on mangel-wurzel var. Golden Globe, red garden beet, and four varieties of sugar beet planted at the Estación Experimental Agrícola de Tucumán. The disease was found also on one plant of a species of *Physalis* and symptoms resembling those of curly top as it occurs in the United States were noted on two plants of cultivated petunia (*× Petunia hybrida* Vilm.).

No symptoms completely typical of curly top were found on tomato, although several small plots of tomatoes were abundantly exposed to infection. One plot in particular was adjacent to a plot of badly diseased mangel-wurzels, as shown in figure 4, and the vector of curly top was abundant on mangel-wurzels throughout October, November, and December, 1940. Since all the mangel-wurzels, and later a high percentage of sugar beets in an adjacent plot, had curly top, conditions for transfer of the virus from mangel-wurzel and beet to tomato



FIGURE 4.—Plot of badly diseased mangel-wurzels adjacent to a plot of tomatoes. Although leafhoppers (*Agalliana ensigera*) were abundant on the mangel-wurzels during most of the season of 1940–41, no curly top was observed on the tomatoes. Photographed at Tucumán, Argentina, December 1940.

appeared excellent. However, none of the tomato plants showed symptoms of curly top at any time.

It is not impossible that strains of curly top virus able to attack tomatoes occur in Argentina despite the fact that none was discovered in the field or through tests with leafhoppers. It seems probable, however, that the disease reported earlier as curly top on tomato is another virus disease that produces symptoms resembling curly top as it is known on tomato in the United States. Fawcett (12) has shown recently that the causal virus of this Argentine tomato disease is transmitted by thrips (*Frankliniella paucispinosa* Moul.).

The tomato disease was present in a number of fields in the vicinity of Tucumán and was found in the lower Río Negro Valley. Leaves of affected plants rolled and turned yellow, and in some instances the plants died. In the early stages, however, there were necrotic spots on the young leaves but veinlet clearing was absent. Fawcett (12) is of the opinion that this disease is a type of spotted wilt.

Attempts by the senior author to transmit the disease from tomato to sugar beet by means of *Agalliana ensigera* resulted in no infection. Juice inoculation from tomato to *Nicotiana glutinosa* L., *N. tabacum* L. var. Turkish, and \times *Petunia hybrida* also resulted in no infection. Fawcett (13) was unable to transmit the disease to *Datura stramonium* L. by means of grafts. Spotted wilt virus is reported to cause local lesions on the first three of these species and to cause systemic infection on *D. stramonium*. The general symptoms on tomato, the properties of the virus as determined by Fawcett, and the vector relationship indicate, however, that it may be a strain of the spotted wilt virus, perhaps different from any yet described from other parts of the world. This virus produces a serious disease (corcova) of tobacco in the Province of Tucumán.

If this disease of tomato is caused by a strain of the spotted wilt

virus, it is of interest to note that what is now believed to be the same trouble was described by Fawcett (11) in 1930, the year that Samuel, Bald, and Pittman (19) reported the virus nature of spotted wilt in Australia. Fawcett's record, therefore, would be evidence of contemporaneous occurrence of spotted wilt in the Western Hemisphere.

PLANTS INFECTED EXPERIMENTALLY

Tests were made on a number of species and varieties of plants to determine their susceptibility to the curly top virus found at Tucumán. In order to provide a better basis for comparing the North American and the Argentine curly top, several plants were selected whose reaction to curly top as it occurs in the United States was already known.

The plants tested were grown in pots under cloth cages. Some of the inoculations were made by means of viruliferous *Agalliana ensigera* reared on diseased beets in cages, and others by means of leafhoppers captured on diseased plants in the field. In general, except on seedling beets on which one leafhopper per plant was used, relatively large numbers of insects were placed on each plant, and most of the plants were inoculated several times by means of insects from different sources. The species and varieties of plants inoculated and the results obtained are shown in table 2.

Infection was obtained on all the beet varieties, but the percentages of infection were not so high as those usually obtained with *Eutettix tenellus* and a virulent strain of North American curly top virus.

No infection was obtained on tomato, although large numbers of leafhoppers were used and the plants were inoculated repeatedly by means of leafhoppers from stock colonies as well as leafhoppers from diseased mangel-wurzels and sugar beets in the field. As reported by Fawcett (11), young leaves of inoculated plants were rolled, especially on plants on which large numbers of leafhoppers were placed. When the leafhoppers were removed, however, the plants produced normal leaves. The variety of tomato (Manzana) used in these tests was later tested at Riverside, Calif., and found to be susceptible to North American curly top.

TABLE 2.—Results of inoculation of different species and varieties of plants with Argentine curly top virus by means of *Agalliana ensigera*

Species or variety inoculated	Leafhoppers caged on each plant	Plants inoculated	Plants infected	
	Number		Number	Percent
<i>Beta vulgaris</i> var. S. L. 68	1	78	8	10
<i>Beta vulgaris</i> var. U. S. 11	1	31	13	42
<i>Beta vulgaris</i> var. U. S. 12	1	21	11	52
<i>Beta vulgaris</i> var. R. & G. Old Type	1	40	16	40
<i>Beta vulgaris</i> var. S. L. 842	1	42	10	24
<i>Nicotiana tabacum</i> var. Turkish	25-500	20	0	0
<i>Lycopersicon esculentum</i> var. Manzana	10-500	29	0	0
<i>X. Peltunia hybrida</i> , Rosy Morn type	50-200	10	0	0
<i>Nicotiana glutinosa</i>	25-100	12	0	0
<i>Datura stramonium</i>	2-50	15	0	0
<i>Datura meteloides</i> DC.	50	10	0	0
<i>Capsicum frutescens</i> L.	15-100	2	0	0
<i>Arachis hypogaea</i> L.	25-100	12	0	0
<i>Physalis</i> sp.	100	2	0	0
<i>Amaranthus</i> sp.	10-100	12	0	0
<i>Stellaria media</i> (L.) Cyr.	10	53	27	51
<i>Zinnia elegans</i> Jacq.	2-100	10	1	10
<i>Phaseolus vulgaris</i> L.	50	11	0	0

Other members of the family Solanaceae also appeared to be resistant or immune. Of the species of this family tested, *Nicotiana tabacum* var. Turkish, *N. glutinosa*, *Datura stramonium*, and \times *Petunia hybrida* are susceptible to North American curly top and *D. meteloides* is resistant or immune. Evidence already mentioned, however, indicates that petunia and a species of *Physalis* sometimes become infected under natural conditions in Argentina.

Attempts were made to infect tobacco by means of grafts. In the first of these, sections cut from stems of two petunia plants that showed symptoms identical with those found on petunia in the United States when infected by curly top were inserted into the stems of five Ambalema tobacco plants. Four of the scions soon died, but the fifth lived for more than 5 weeks and produced a small amount of growth. No symptoms were produced on any of the inoculated plants. In a second test, sections of the stem of a plant of *Physalis* sp., known to have curly top virus through tests with leafhoppers, were grafted into the stems of five Turkish tobacco plants. The scions remained alive for more than 3 weeks, but no infection was produced.

Zinnia elegans appears to be very resistant to infection, since only 1 of 10 plants, each inoculated by means of large numbers of leafhoppers, became infected. In other tests leafhoppers placed on large numbers of seedlings in the open produced no infection. Chickweed (*Stellaria media*) appears to be relatively susceptible, although infection was low as compared with that normally obtained with North American curly top virus.

DESCRIPTION OF SYMPTOMS ON VARIOUS HOSTS

SUGAR BEET

After the inoculation of cotyledon-stage sugar beets with Argentine curly top by means of *Agalliana ensigera*, symptoms appeared on the young leaves in 4 to 10 days. The first symptoms consisted of veinlet clearing, vein swelling, and leaf rolling. In some cases, rolling and twisting of leaves were very marked even on the varieties U. S. 11 and U. S. 12, resistant to North American curly top. However, severely diseased seedlings were not killed and as they continued to grow the symptoms became progressively less severe. Figure 5, A, shows plants of the varieties R. & G. Old Type, U. S. 12, and U. S. 11 inoculated in the seedling stage but partially recovered. U. S. 12 and U. S. 11 show the most marked evidence of recovery; the symptoms on R. & G. Old Type are still rather severe. Figure 5, B, shows the plants at a later stage.

Plants inoculated when they had 8 to 10 true leaves also showed severe primary symptoms. The first affected leaves were curled, veinlets were translucent, and the petioles were shorter than on healthy plants. Phloem exudate was abundant on the petioles and main veins of the leaves in many cases. Later growth was more normal in appearance and, with the exception of the varieties R. & G. Old Type and S. L. 842, all recovered to such an extent that they showed only veinlet clearing in the younger leaves and slight vein swelling in the older ones. Even on R. & G. Old Type and S. L. 842, the leaves were almost normal in size and shape, but vein swelling was conspicuous and protuberances and spiny outgrowths from the veins were common. This type of recovery was characteristic of the



FIGURE 5.—A, Sugar-beet plants, severely affected with Argentine curly top after inoculation in the cotyledon stage, showing distinct evidence of recovery: a, R. & G. Old Type; b, U. S. 12; c, U. S. 11. B, Same plants at a still later stage of development. U. S. 12 (b) and U. S. 11 (c) have almost completely recovered from the effects of the disease; R. & G. Old Type (a) shows only veinlet clearing and vein swelling. Photographed at Tucumán, Argentina, 1941.

regular course of the disease on sugar beet as it was observed on plants in pots and in the field. In contrast, plants affected with North American curly top, at least those of susceptible varieties, show little or no evidence of recovery after infection.

In recently infected beets, phloem necrosis was as characteristic and marked in plants affected with Argentine curly top as it is in those

affected with North American curly top. Necrosis was especially evident in the petioles and larger veins of recently infected plants. Microscopic examinations of transverse sections of petioles revealed blackened phloem areas in which many of the cells had collapsed. Later there was evidence of tissue regeneration similar to that described by Esau (7) in plants affected with North American curly top. Phloem necrosis was marked in roots of all ages in the field and under cages. In the larger beets rings of blackened phloem were evident but rings produced as the plants recovered were less conspicuous. Severity of vascular necrosis appeared to be more or less correlated with severity of leaf symptoms.

MANGEL-WURZEL

Diseased mangel-wurzels of the Golden Globe variety, planted and inoculated by G. L. Fawcett, were available for study in October 1940. At that time the plants were small to medium, with roots 1 to 3 inches in diameter, and gave evidence of having been infected when relatively small. There was much variation in severity of symptoms on the different plants. Some were severely stunted, with leaves rolled and crinkled and petioles shortened. Exudate was present on the petioles of many of the younger leaves. When the plants were removed from the soil, it was observed that many of the rootlets had died and others had pushed out, producing a bunched appearance similar to that found frequently on beets affected with curly top in the United States. In some plants transverse sections of the roots revealed dark rings marking necrosis in the vascular regions. Vascular necrosis appeared to be more marked in the region of the crown than in other parts of the root, but in the specimens examined necrosis was not excessive and in many specimens it was not clearly evident. Necrosis was observed also in the region of the phloem in petioles of the more severely affected leaves.

Some of the plants were mildly affected and appeared normal in size and general appearance. All these plants, however, showed more or less distinct vein swelling on the older leaves and veinlet clearing on the younger ones. Usually, necrosis was not evident in the phloem of roots and petioles of plants of this type. Between these two extremes there were many plants showing various degrees of injury.

PETUNIA

On two plants of petunia believed to be naturally affected with curly top, the leaves were yellow and curled. Some were distorted and twisted, and the younger ones showed distinct veinlet translucency. Veins of older leaves were conspicuously swollen. Vein swelling and distortion of tissues were evident also on the corolla and calyx. The plants eventually yellowed and died.

PHYSALIS

The young leaves of all the growing points of a large physalis plant found naturally infected were distinctly curled and crinkled. The youngest leaves had clearly defined translucent veinlets. Growth of all shoots was much retarded.

ZINNIA

Primary symptoms on zinnia consisted of veinlet clearing and rolling of the younger leaves, followed by vein swelling. Growth of the terminal of the one plant observed was much reduced, but the plant eventually flowered (fig. 6). The first blossom was small, the color was

FIGURE 6.—Zinnia plant infected with Argentine curly top by exposure to viruliferous *Agalliana ensigera* at Tucumán, Argentina.



partly suppressed, and the petals were reduced in size and distorted and showed distinct vein swellings. Lateral buds grew into shoots that were at first much distorted, with leaves small and curled. The shoots later recovered to a considerable degree and the blossoms were more nearly normal.

CHICKWEED

Only the early stages of the disease on chickweed were observed. Young leaves developed distinct veinlet clearing in periods of from 5 to 8 days after inoculation. As the leaves of affected plants continued to grow they became puckered or curled and twisted. Prominent swollen areas were produced on the veins. The tips of the shoots became distorted and frequently turned downward, and growth was much retarded.

VARIETAL RESISTANCE OF SUGAR BEETS

Limited tests were made to determine the relative resistance of the sugar-beet varieties S. L. 68, U. S. 11, and U. S. 12, known to be resistant to North American curly top, and R. & G. Old Type and S. L.

842, known to be very susceptible to North American curly top. In the tests with potted plants of these varieties, seedlings were inoculated by placing one viruliferous *Agalliana ensigera* on each plant and allowing the insects to feed for 3 days. All inoculated plants were kept in cloth cages until final results were recorded. The results of a series of tests are shown in table 3.

TABLE 3.—Results of tests to determine relative susceptibility of varieties of sugar beet to Argentine curly top

Test No.	Plants inoculated ¹		Plants infected	
	Variety	Number	Number	Percent
1.	S. L. 68.	19	2	11
	R. & G. Old Type	15	4	27
2.	U. S. 11	12	7	58
	U. S. 12	12	5	42
3.	S. L. 68.	38	5	13
	S. L. 842	21	4	19
4.	S. L. 68.	21	1	5
	U. S. 11	19	6	32
	U. S. 12	19	6	32
	R. & G. Old Type	25	12	48
	S. L. 842	21	6	29

¹ All plants were inoculated in the cotyledon stage by placing 1 viruliferous leafhopper on each plant and allowing it to feed for 3 days.

The same varieties, except S. L. 842, were planted for the field tests October 24 in a plot adjacent to rows of mangel-wurzels infected with curly top and heavily infested by *Agalliana ensigera*. Germination of seed was excellent, and an almost perfect stand of plants was obtained. Leafhoppers began to migrate to this planting as soon as the first seedlings were through the soil, and leafhoppers continued to be present in considerable numbers throughout the time the plot was under observation. Symptoms of curly top began to appear on many of the plants with the production of the first true leaves. The disease continued to spread, and soon after the plants were thinned on November 24 all varieties, except S. L. 68, showed a high percentage of infection. The results of counts of diseased plants made on different dates are shown in table 4.

In the earlier stages of the disease, U. S. 11 showed the highest percentage of infection, probably because it was closest to the badly diseased mangel-wurzels that served as the principal source of infection.

TABLE 4.—Percentage of curly-top-diseased plants found in varieties of sugar beet in plots at Tucumán, Argentina, on the indicated dates

[Beets planted October 24, 1940]

Variety	Plants showing symptoms of curly top on—					
	Nov. 12	Nov. 18	Dec. 2	Dec. 10	Jan. 7	Feb. 8
	Percent	Percent	Percent	Percent	Percent	Percent
U. S. 11	5	22	68	95	100	81
R. & G. Old Type	1	9	56	94	100	100
S. L. 68	Trace	Trace	8	17	82	43
U. S. 12	Trace	1	41	86	100	89

The early symptoms were severe on seedlings of all varieties. Leaves were much distorted, and the younger ones showed marked veinlet translucency. Symptoms were almost as severe on U. S. 11 and U. S. 12 as on R. & G. Old Type, but they were somewhat less severe from the beginning on S. L. 68. Symptoms continued severe until thinning time, November 24. After the plants were thinned, growth was very rapid and by December 16 there was a marked difference in the appearance of the different varieties. Most of the plants of S. L. 68 had recovered to an appreciable degree, the symptoms consisting of slight vein swelling and in some cases a tendency toward crinkling of leaves. U. S. 11 and U. S. 12 also had recovered to a marked degree. The three rows of R. & G. Old Type were beginning to recover, but the foliage was somewhat yellower than that of U. S. 11 and U. S. 12 and more of the lower leaves of the plants of this variety were dying. On December 17 a hot wind blew for several hours, and it was observed that the plants of R. & G. Old Type wilted more than those of the other varieties. However, a photograph taken December 16 (fig. 7) showed little difference in the size of plants of the different varieties.



FIGURE 7.—Plot at Tucumán, Argentina, in which North American varieties of sugar beet were tested for resistance to Argentine curly top. At the extreme right, and only partly in view, are large plants of diseased mangel-wurzels (*a*) that furnished the inoculum for the sugar beets. U. S. 11 (*b*), resistant to North American curly top, occupied three rows to the left of the mangel-wurzels. The next three rows to the left are R. & G. Old Type (*c*), susceptible to North American curly top; farther to the left are the resistant varieties (*d*) U. S. 12 and S. L. 68. In this stage, plants of the different varieties differed little in size. However, symptoms consisting of veinlet clearing, vein swelling, slight leaf rolling, and yellowing of the older leaves were most marked in R. & G. Old Type plants. Under similar conditions of infection with North American curly top, the difference in size between resistant and susceptible plants would be much greater. Photographed December 16, 1940.

By January 7, after 2 weeks of hot, dry weather, approximately 20 percent of the plants of all varieties had died. Plants apparently healthy wilted suddenly and when pulled were found to have rot on the lower portion of the main root. This trouble was apparently distinct from curly top, but the cause was not determined.

At this time, R. & G. Old Type definitely showed more severe symptoms of curly top than the other varieties. The petioles of plants of this variety were slightly but not markedly shortened, the leaves were rolled in some plants, and protuberances and spiny outgrowths were common. There was considerable variation in the severity of the disease on different plants, and this was true also of U. S. 11 and U. S. 12. There was little difference in the average size of plants of the different varieties.

During January these plants were severely attacked by leaf spot caused by *Cercospora beticola* Sacc., and by February 8 many of the older leaves had been killed by this disease. However, the plants had continued to recover from the effects of curly top. Symptoms were indistinct on S. L. 68, and many of the infected plants showed no clearly defined symptoms. This was true also for U. S. 11 and U. S. 12, but to a lesser degree. Certain plants of these latter varieties had clearly defined but rather mild vein swelling, but there was no evidence that the disease had interfered appreciably with growth. Vein swelling and general roughness of leaves were evident on most of the plants of R. & G. Old Type, but there was little evidence of production of leaves with short petioles or of stunting of the plants. Typical diseased plants of R. & G. Old Type and of U. S. 11 are shown in figure 8.

The results of observation of the plants in this plot, together with those made on seedling beets in pots, indicate that of the varieties tested S. L. 68 showed the most resistance, followed by U. S. 11 and U. S. 12. R. & G. Old Type was the most susceptible. This is the order in which these varieties stand in their resistance to curly top in the United States.

THE VECTOR

CHARACTERISTICS, DISTRIBUTION, AND HOST RANGE

The leafhopper *Agalliana ensigera*, vector of the curly top virus in Argentina, is about the size of *Eutettix tenellus*, vector of North American curly top virus, which it resembles in many of its characteristics and habits. The males are smaller and darker than the females. The nymphs hatch from eggs in about 9 days at a temperature of 100° F. and develop into adults in about 25 days at this temperature. Like *E. tenellus*, this insect has a high temperature optimum for development. The nymphs are somewhat more sluggish than those of *E. tenellus*, and when disturbed usually run to the opposite side of the stem or leaf instead of jumping. Adults usually fly when disturbed. When confined in cages or tubes, they often make a distinctly audible buzzing sound with their wings.

The favorite host plant appears to be the beet. Eggs are laid in beet plants in great numbers, and colonies totaling a thousand or more individuals may be reared on a single large plant. In the Province of Tucumán, the insect was found to be abundant where beets of any



FIGURE 8.—Reaction of two varieties of sugar beet to Argentine curly top. The plants were of the same age and were similarly inoculated. *A*, R. & G. Old Type, showing vein swelling and rugose leaves; *B*, U. S. 11, showing almost no symptoms of curly top. Photographed at Tucumán, Argentina, 1941.

kind were growing. In the vicinity of beet plantings, the insect was found on two species of *Amaranthus*, on one species of *Portulaca*, and on *Datura stramonium*, *Zinnia elegans*, and *Chenopodium album* L. It was not found on any of the native shrubs or on plants outside the cultivated areas.

In the lower Rio Negro Valley the leafhopper was very scarce during the month of February 1941, but small numbers were found on sugar beets. In this region plants of Russian-thistle (*Salsola kali* L. var. *tenuifolia* Tausch.), alfalfa (*Erodium cicutarium* L'Hér.), *Chenopodium* spp., and mustards were abundant in the plantings of beets and in areas adjacent. Apparently none of these harbored the insect to any appreciable extent. The leafhopper was not captured on any of the native vegetation outside the cultivated area.

The leafhopper breeds readily on mangel-wurzel and red garden beet as well as on sugar beet. It is able to breed also on *Amaranthus* spp., *Datura stramonium*, *D. meteloides*, and *Zinnia elegans*, and to a limited extent on *Chenopodium album*. Of these plants, only mangel-wurzel, beet, and *Amaranthus* spp. appeared to be important in maintaining the leafhopper population observed at Tucumán. Apparently the insect is unable to breed on tobacco and tomato.

The native hosts of *Agalliana ensigera* in Argentina are unknown. The beet is an introduced plant and is grown to only a very limited extent in the Province of Tucumán. It seems probable that it is of little importance in determining the survival of the leafhopper, though it may be of considerable importance in bringing about great local increases in populations of the insect. It seems probable that the natural host plants may not be sufficiently favorable to support a large population of the insect but are able to provide only for its survival at a relatively low population level. Obviously, a more complete knowledge of the host range of *A. ensigera* might open new avenues for study of the reservoirs of the curly top virus in Argentina.

FEEDING HABITS IN RELATION TO CURLY TOP TRANSMISSION

Although *Agalliana ensigera* may be found in feeding position on all exposed parts of the beet plant, it is commonly found on the smaller leaves and along the two edges of the petioles of the larger leaves. Evidence indicates that it feeds by preference on the vascular bundles.

Microscopic examination of transverse sections of petioles on which large numbers of leafhoppers had fed revealed many clearly evident feeding punctures. These were marked by yellowish deposits of saliva. In many cases the line of puncture extended from the epidermis directly to a vascular bundle and terminated in the phloem. Deposits of salivary material were evident in the phloem and in tissues outside the phloem.

The punctures extended directly through the cell walls and protoplasmic content, but the punctured cells appeared to suffer no immediate ill effects. The lines of puncture were straight except where they curved to enter the vascular bundles. They were marked by thin and not always continuous sheaths of salivary material introduced by the leafhoppers. Judging from the sections of beet petiole examined, it seems probable that *Agalliana ensigera* introduces less salivary material into the plant than does *Eutettix tenellus* under

similar conditions of feeding. When the leafhopper fed on the petioles of young leaves, drops of clear liquid often appeared above the punctures after the mouth parts were withdrawn. Possibly this may have been due to the introduction of saliva insufficient to plug effectively the puncture made in feeding. If the lines of puncture were plugged incompletely by coagulated saliva introduced by the insect, it might be expected that, since the phloem content is under a positive pressure, liquid content of the phloem would flow out through the holes made by the mouth parts of the insect. This might be true especially if the sheath passing through the parenchyma were more or less complete and if the end entering the phloem were open.

In a study of some of the properties of the virus the ability of the leafhopper to feed on liquids through a membrane was tested before methods of artificial feeding were used. The first tests were made with drops of sugar solution placed on the outer surface of Baudruche capping skin covering one end of a cage in which the insects were confined. A rather thick grade of capping skin was used in these tests. The leafhoppers were able to locate the drops of sugar solution, but it was evident that they had great difficulty in penetrating the capping skin. After finding the drops they extended their legs as if to obtain a firmer footing, placed their proboscises in contact with the membrane, and moved their heads as if attempting to force the mouth parts through the membrane. With the thinner capping skin employed later, it was found that some of the leafhoppers were able to penetrate the membrane. In a few instances, mouth parts could be seen clearly in the drops of sugar solution below the membrane. However, the leafhoppers were restless and the mortality in a 2- to 4-hour period at 100° F. (37.8° C.) was very high.

In further tests, the capping skin was replaced by lens paper lightly impregnated with paraffin. The leafhoppers fed much more readily through this type of membrane than through capping skin. For best results, however, it was necessary to have just enough paraffin to prevent wetting of the paper but not enough to completely fill all the interstices. The leafhoppers did not readily penetrate paraffin films but fed through openings in the paper not closed by the paraffin. With this kind of membrane, the leafhoppers fed reasonably well and, with most of the food materials used, remained alive throughout a feeding period of 4 to 6 hours at a temperature well above 100° F.

When the setae of *Eutettix tenellus* penetrate a membrane and enter a liquid, a salivary secretion flows out of the tips of the setae, immediately gels, and forms a sheath around the mouth parts. Usually, by repeated probing and emission of more salivary secretion, an appreciable deposit of coagulated material is built up. Deposits of this type are visible under low magnification and may be seen also in clear liquids by the unaided eye.

Drops of sugar solution on which individuals of *Agalliana ensigera* were feeding were watched under a hand lens, and penetration of the drops by the mouth parts of many leafhoppers was observed. These observations, however, did not reveal the presence of any coagulable material in the vicinity of the mouth parts. When the setae entered the liquid they came through the membrane free of any indication of introduced material, and throughout the feeding periods the setae remained naked in the liquid. Moreover, no deposits of salivary

material were found in drops on which the leafhoppers had fed for 4 to 6 hours. As already stated, however, deposits of salivary secretions were found in the plant tissues in which the leafhopper fed. This of course shows that *A. ensigera* does produce salivary secretions that are emitted and that coagulate in plant tissue. Failure to observe such deposits in sugar solutions indicates that for some reason the leafhopper introduces little, if any, coagulable salivary secretions when feeding on such liquids.

The evidence indicates that leafhoppers introduce the virus into plants through the medium of the coagulable saliva. This has not been proved definitely, but it is significant that in the case of all leafhopper vectors on which information is available coagulated salivary secretions have been found in the infected plants. Smith (21) showed that liquids on which *Eutettix tenellus* fed contained free curly top virus that could be picked up by nonviruliferous leafhoppers. Whether this was introduced into the liquids free of the coagulable saliva or was introduced with it and later diffused out of the coagulum into the surrounding medium was not determined.

Since *Agalliana ensigera* apparently does not introduce coagulable saliva into sugar solutions, experiments were made to determine whether this insect also fails to introduce virus into such solutions. In these tests viruliferous individuals were allowed to feed on drops of sugar solution through a membrane for about 1 hour. The drops were then transferred to a second membrane through which non-viruliferous leafhoppers fed for about 4 hours. The leafhoppers of this second lot were then caged singly on seedling beets. The results of these tests are presented in table 5, together with results of similar tests made at Riverside, Calif., with *Eutettix tenellus* and the North American curly top virus. It may be noted that no infection was obtained from the liquids on which viruliferous *A. ensigera* had fed, whereas relatively high percentages of infection were obtained from liquids on which viruliferous *E. tenellus* had fed.

TABLE 5.—Results of tests to determine whether viruses of North American curly top and Argentine curly top are introduced into sugar solutions during the feeding of viruliferous individuals of *Eutettix tenellus* and *Agalliana ensigera*, respectively

Species of leafhopper tested	Test No.	Plants inoculated ¹	Plants infected	
			Number	Percent
<i>Agalliana ensigera</i> 2	1	12	0	0
	2	14	0	0
	3	6	0	0
<i>Eutettix tenellus</i> 3	1	20	11	55
	2	19	9	47
	3	20	14	70

¹ Nonviruliferous leafhoppers of each species, fed on sugar solutions on which viruliferous leafhoppers of the same species had previously fed, were allowed to feed on the test sugar-beet plants.

² Tested at Tucumán, Argentina, November 1940.

³ Tested at Riverside, Calif., April 1939.

Although the tests with *Agalliana ensigera* are somewhat limited, the apparent absence of virus from the liquids in which no coagulated salivary deposits were observed is further indication that the introduction of virus into a medium by a leafhopper is associated with the introduction of coagulable salivary secretions. If virus is introduced into plants directly with the coagulable saliva the quantity of virus

would tend to be proportional to the amount of saliva introduced in insect feeding. Since it seems probable, on the basis of observations so far made, that *A. ensigera* introduces less coagulable saliva into the plant than does *Eutettix tenellus*, this may be a factor influencing adversely the efficiency of *A. ensigera* as a vector.

THE VIRUS

INCUBATION PERIOD IN VECTOR

The period that must elapse between the time *Eutettix tenellus* begins to feed on a diseased plant and the time it is able to transmit the virus of North American curly top to a healthy plant has been found to be as short as 4 hours in some instances. Assuming that the incubation period of the Argentine curly top virus in *Agalliana ensigera* also would be short, preliminary experiments were made in which nonviruliferous leafhoppers were allowed to feed on a diseased plant for 2 hours and were then transferred at hourly intervals to sugar-beet seedlings. When these tests gave no infection, the time the leafhoppers were allowed to feed on the diseased plants and on the seedlings was increased. The results of six tests are shown in table 6.

The infection obtained was less than would be expected with North American curly top virus and *Eutettix tenellus*, but the number of plants inoculated probably was not large enough to provide an accurate estimate of the minimum incubation period of the Argentine curly top virus in its vector. However, the fact that infection was usually produced by insects that had fed from 24 to 72 hours shows that the incubation period of the virus in the insect is relatively short. More extensive tests are needed to determine whether in certain individual insects it may be considerably shorter than that indicated by the results obtained in this series of tests.

PERIOD OF RETENTION IN VECTOR

On November 12, 1940, about 200 large nymphs and young adults of *Agalliana ensigera* were removed from a plant of sugar beet affected with Argentine curly top, placed on a vigorous plant of *Chenopodium album*, and allowed to remain there until December 18. By that time mortality was high, but 11 adults of the leafhoppers originally introduced were recovered and caged singly on seedling sugar beets. By January 3, 5 of the 11 plants had become diseased, clearly indicating that the leafhoppers still carried the virus. When the adult leafhoppers were removed from the *C. album* plant, it was found that a number of half-grown and smaller nymphs were present that had developed from eggs laid by the original viruliferous leafhoppers. Of these, 32 were caged on 16 seedling sugar-beet plants. None of these plants developed symptoms of curly top. Since nymphs developed from eggs deposited on *C. album* were unable to obtain virus by feeding on its juices, it appears that this plant is immune to curly top. This being true, the viruliferous adult leafhoppers placed on the plant November 12 did not increase their virus content through feeding and, since they still contained virus on December 18, it is evident that *A. ensigera* is able to retain the virus of Argentine curly top in its body for at least 36 days. It is also evident that the virus does not pass through the egg stage of the insect.

TABLE 6.—Incubation period of Argentine curly top virus in *Agalliana ensigera*

Test No.	Period of leaf-hopper feeding on diseased beets	Number of sugar-beet plants inoculated and infected by leafhoppers in which the virus had the indicated incubation period (hours)																													
		3		4		5		6		7		8		24		48		72		96		120		144		192		240			
		Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
1	Hours	3	0	4	0	3	0	4	0	2	0	3	0	1	0													3	0		
2	2													5	1	5	0			3	2										
3	3													4	0	4	1			4	2			5	2			3	1		
4	24													4	0	4	1														
5	24																	3	0												
6	4									19	0			16	0	10	1	17	1									4	2		

OCCURRENCE IN PHLOEM EXUDATE

In view of the fact that exudate from the phloem of diseased beets has proved to be the most satisfactory medium from which to recover the virus of North American curly top, tests were made to determine whether the curly top virus in Argentina could also be recovered from phloem exudate. Small quantities of exudate produced naturally on the petioles of diseased plants were collected by means of a capillary tube. The exudate was diluted about 1 part to 9 parts of distilled water and fed to nonviruliferous leafhoppers. After a feeding period of about 6 hours, the leafhoppers were caged singly on seedling beets. Of 12 plants inoculated, 4 became infected.

PROPERTIES

Phloem exudate⁸ diluted 1 part of exudate to 9 parts of distilled water was used in a series of tests to determine the thermal inactivation point of the virus. The diluted exudate was placed in small test tubes (about 2 cc. per tube). The tubes were then covered with rubber caps and placed in a water bath held as nearly as possible at the desired temperature for 10 minutes. After treatment the tubes were removed from the bath and placed immediately in cold water. Non-viruliferous leafhoppers were allowed to feed on the treated exudate for about 6 hours and were then caged singly on seedling sugar beets. The results of three tests are shown in table 7, together with the results of one test in which alcoholic precipitate from viruliferous leafhoppers was used as the source of virus.

Infection was relatively low in all tests. For this reason the results are not so conclusive as they would be had higher percentages of infection been obtained. The results indicate, however, that the thermal inactivation point of the virus lies between 75° and 80° C. This is also the range in which the curly top virus of North America is inactivated.

TABLE 7.—Results of tests to determine the thermal inactivation point of virus of Argentine curly top

Test No.	Number of sugar-beet plants inoculated and infected by means of leafhoppers that fed on virus suspensions heated for 10 minutes at the indicated temperature (°C.)													
	Check, not treated		55		60		65		70		75		80	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
1	10	1			10	0			10	0				
2	12	6	11	6	8	1	10	4						
3 ¹	3	0					7	1	5	0	8	2	5	0
4	16	2					16	1	19	1	20	1	17	0

¹ The source of virus in this test was a wash of alcoholic precipitate of macerated, viruliferous leafhoppers.

⁸ Tests were made with the exudate from the cut surface of large diseased roots of mangel-wurzel. To obtain exudate, the roots were taken from the field and placed in pails of water in the laboratory. Cuts were made across the crowns of the roots to induce flow of liquid from the phloem. In most cases the roots did not exude appreciably the first day but on the second, third, and fourth days flow was more abundant. In some cases it was possible to collect as much as a cubic centimeter of exudate from lots of six or more roots. This exudate was used for further studies of the virus.

Dilution tests were made in which phloem exudate was diluted with distilled water, 1 part of exudate in 10 parts of the mixture; 1 part of exudate in 100 parts; and 1 part of exudate in 1,000 parts. Nonviruliferous leafhoppers were allowed to feed on these mixtures for about 6 hours and then were caged singly on seedling sugar beets. From each dilution 12 plants were inoculated. Only 1 plant became infected, and this was by a leafhopper that had fed on the 1-in-10 dilution.

Attempts were made to recover virus from different types of precipitates. Juice from diseased beets was precipitated in 50-percent alcohol. The precipitate was recovered by filtration, dried at laboratory temperature, and mixed with 5-percent sugar solution. The filtrate from this was fed to nonviruliferous leafhoppers that were later caged singly on seedling sugar beets. Tests were made with fresh beet juice and with juice held at laboratory temperatures for 1, 2, and 3 days. No infection was produced by leafhoppers that fed on these mixtures.

In tests involving also thermal inactivation, essentially the same technique as that described above for virus recovery was used but the source of virus was macerated, viruliferous leafhoppers. Infection was obtained from a wash of the alcoholic precipitate of macerated leafhoppers. These results are given in test 3 of table 7. The heat treatments superimposed on the wash are not interpreted as having significant influence on the release of virus.

Phloem exudate, dried and kept at laboratory temperatures, was tested for virus content at intervals of 2 weeks over a period of 2 months. The exudate in these tests gave little infection when fresh, and no virus was recovered from the dried material.

TRANSMISSION BY MECHANICAL INOCULATION

Sugar-beet plants of the variety U. S. 11, having about six true leaves and growing rapidly, were inoculated by needle with phloem exudate as a source of virus. The exudate was obtained as clear drops from the surface of petioles of recently infected sugar-beet plants and was placed in the axils of the leaves at the crowns of the healthy beet plants. Numerous punctures were made with a very fine needle through these drops of exudate into the crowns of the plants. Of 20 plants inoculated in this manner 4 became infected. Twenty noninoculated plants held as controls remained healthy.

In a second type of test, phloem exudate was collected from diseased mangel-wurzels, diluted about 1 part of exudate to 4 parts of distilled water and rubbed over the surface of leaves of rapidly growing sugar beets of the variety U. S. 11. Leaves were inoculated with and without the use of an abrasive. Thirty-seven plants were inoculated, but no infection resulted.

More extensive tests are needed to determine whether a relatively high percentage of infection could be obtained consistently by needle inoculation. The same technique has given infection with the virus of North American curly top, but the percentage of infection, with few exceptions, was very low. No infection with North American curly top virus has been obtained in extensive tests in which inoculum was rubbed over the surface of leaves.

RATE OF MOVEMENT IN BEET LEAVES

Twenty-four numbered pots, each containing two rapidly growing beet plants having about eight true leaves each, were used in a test to determine the rate of movement of the curly top virus out of inoculated leaves. These plants had grown in semishade and each plant had at least one leaf longer than 15 cm. Ten viruliferous leafhoppers were caged on the tip of one of the longer leaves of each plant at a temperature somewhat above 100° F. (37.8° C.) for 2 hours. At the end of the 2-hour feeding period, the inoculated leaves of all the plants in pots with even numbers were severed at a distance of 15 cm. from the part on which the leafhoppers fed. At the same time the leafhoppers were removed from the leaves of the plants in pots with odd numbers. This second group of plants was retained as a check on the amount of infection produced by the feeding of the leafhoppers.

Three of twenty-four plants from which the leaves were removed and 4 of 24 plants from which the leaves were not removed became infected. This result indicates that the virus is able to move downward from the point of inoculation at a very rapid rate, the measured movement being at least 15 cm. in 2 hours.

DISCUSSION

RELATIONSHIP OF ARGENTINE AND NORTH AMERICAN VIRUSES

The considerations of major interest and importance in relation to the Argentine curly top disease are (1) its relationship to North American curly top and (2) its potential capacity for injury if introduced into the sugar-beet areas of the United States and other parts of the world.

Certain differences in characteristics of curly top in the two continents are evident, but these differences are not extensive. The vector of the North American curly top virus did not transmit the Argentine virus. The Argentine virus so far as is known does not infect tomato and tobacco. However, Giddings (14) has shown that certain strains of the North American virus also do not infect tomato and tobacco. In general, the disease caused by the Argentine virus is characterized by more severe curling and distortion on seedlings of varieties resistant to North American curly top, but infected plants of both resistant and susceptible varieties recover to a marked degree. No comparable recovery has been noted in beet plants infected by the North American virus, especially in those of susceptible varieties.

The similarities between the two diseases are very striking. The symptoms are almost identical on all host plants on which the two diseases have been observed. All varieties of sugar beet developed for resistance to the North American virus that were tested were found to be resistant to the Argentine virus. In general, except for certain members of the Solanaceae, the host range of the two viruses, so far as known, is the same. Properties of the two viruses, insofar as they have been determined, are the same or very similar.

The occurrence of two similar diseases in regions so widely separated as the United States and Argentina, in the absence of any evidence of a common origin, leaves the question of their relationship

rather difficult to determine. At one time, the fact that the vector of the North American virus does not transmit the South American virus would have been accepted as strong evidence that the viruses of the two continents are distinct and separate in spite of the close similarity of symptoms and host range. However, Black's finding (2, 3) that there are two strains of the potato yellow dwarf virus each transmitted by a different leafhopper weakens the confidence that can be placed in differences with respect to the insect vector in the determination of relationships of viruses.

It may be suggested that superimposition of the North American virus on the Argentine virus indicates that the two viruses are unrelated, since it has been maintained that with certain mosaic viruses complete invasion of a plant by one virus confers immunity from infection by a related virus but not by an unrelated virus. However, Carsner (5), using sugar-beet plants infected by an attenuated strain of the curly top virus, was able to reinfect them by a more virulent strain, with the result that the plants became more severely diseased. Giddings (15), working with 7 clearly differentiated strains of the North American virus, found that beet plants infected for at least a month by one strain of the virus were susceptible to infection by a second strain in 15 combinations tested. Less virulent strains infected plants previously infected by more virulent strains, and more virulent strains were readily introduced into plants already infected by those less virulent. Therefore, the fact that plants infected by Argentine virus remained susceptible to infection by North American virus cannot be accepted as evidence of unrelatedness unless the highly improbable assumption is made that all the strains of North American virus tested are not strains of one virus but are so many separate and distinct viruses.

Because of the marked similarities between curly top of Argentina and that of North America and the wide differences between the characteristics of curly top and all other virus diseases, it seems logical to assume, for the present at least, that curly top of sugar beets in the Western Hemisphere is caused by a single virus complex consisting of different strains and varieties and to classify the Argentine virus as a variety of this curly top virus complex.

In the Latin system of virus nomenclature suggested by Holmes (17), the curly top virus is given the name *Chlorogenus eutetticola* H. Carsner and Bennett (6), however, suggested that the species be shifted to the genus *Ruga* of Holmes on the basis of symptoms induced and that the species name be changed to *verrucosans*. The variety name *distans*, meaning distant, is suggested as a distinguishing epithet for the Argentine virus. The following description of this virus is presented.

PROPOSED NAME AND DESCRIPTION OF ARGENTINE VIRUS

Ruga verrucosans C. and B. var. **distans** n. var.

Virus causing a disease of sugar beet (*Beta vulgaris* L.), red garden beet, chard, mangel-wurzel, chickweed (*Stellaria media* (L.) Cyr.), and zinnia (*Zinnia elegans* Jacq.). Probably most species of Solanaceae, including tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.), very resistant or immune, but typically diseased specimens of *Petunia* sp. and *Physalis* sp. have been found. Disease on *Beta* spp. characterized by leaf curling, veinlet clearing,

vein swelling, vein roughening, and necrosis of phloem. Recovery of sugar beets more marked with Argentine than with North American virus. Transmitted in nature by *Agalliana ensigera* Oman but not by *Eutettix tenellus* (Bak.). Under certain conditions transmissible to small percentage of plants inoculated by needle; not transmissible by rubbing technique. Remains active in insect vector at least 36 days; does not pass through egg stage of vector. Not inactivated by short exposure to 50-percent alcohol. Thermal inactivation point probably between 75° and 80° C.

Type locality.—Province of Tucumán, Argentina.

SIGNIFICANCE OF VIRUS TO OTHER SUGAR-BEET-GROWING COUNTRIES

Although, so far as known, the Argentine curly top virus is limited in its distribution to the northern half of Argentina, the chances of spread of the virus to other countries, through introduction of the vector or of diseased material or of both, have greatly increased in recent years as a result of the development and extensive use of facilities for more rapid transportation.

The economic consequences of the introduction of the Argentine curly top virus into other sugar-beet-producing countries cannot be predicted accurately because of the impossibility of judging, among other things, its effect on all of the possible host plants under a variety of climatic conditions. However, evidence seems ample to justify the assumption that on sugar beet a high degree of control of the disease could be obtained in all sugar-beet-producing areas by using varieties of sugar beet that have been developed in the United States for resistance to the North American curly top.

SUMMARY

Studies of the Argentine curly top disease of sugar beet were made at Arlington, Va., in 1927 and 1937–39, on diseased material from Argentina. Further studies of the disease were made at the Estación Experimental Agrícola de Tucumán in Argentina in the period from September 1940 to March 1941.

Symptoms on Argentine plants grown in the quarantine greenhouse at Arlington, Va., were similar to those on plants affected with North American curly top. However, *Eutettix tenellus*, the vector of the North American curly top virus, failed to transmit the Argentine virus.

In the Province of Tucumán, Argentina, curly top was found on sugar beet, red garden beet, mangel-wurzel, chard, *Physalis* sp., and probably on \times *Petunia hybrida*. It was transmitted experimentally to chickweed, zinnia, and several varieties of sugar beet, but not to any member of the family Solanaceae, including tomato, Turkish tobacco, *Nicotiana glutinosa*, Ambalema tobacco, red pepper, and petunia.

Symptoms of the disease on sugar beet, red garden beet, mangel-wurzel, chard, chickweed, and zinnia, consisting of veinlet clearing, vein swelling, vein protuberances, leaf rolling, and phloem necrosis, were similar to those produced by North American curly top on these plants. In general, symptoms of Argentine curly top were more severe on seedling sugar beet than those of North American curly top, especially in the case of varieties resistant to the North American virus. However, seedlings affected with the Argentine curly top

recovered to a marked degree from the initially severe effects of the disease. Plants that had passed through the recovery phase showed mild to almost no symptoms of disease, depending on the resistance of the variety. Sugar-beet plants affected with North American curly top have not been observed to recover to any appreciable extent.

In tests involving potted plants in cloth cages and in field plots, the varieties S. L. 68, U. S. 11, and U. S. 12, known to be resistant to North American curly top, were found resistant also to Argentine curly top. The variety R. & G. Old Type, very susceptible to North American curly top, was found relatively susceptible to Argentine curly top.

On the grounds of the Estación Experimental Agrícola de Tucumán, the vector of Argentine curly top, *Agalliana ensigera*, was abundant through the season 1940-41 on mangel-wurzels and sugar beets and was found in smaller numbers on *Amaranthus* spp., *Portulaca* sp., *Datura stramonium*, *Zinnia elegans*, and *Chenopodium album*. It was apparently very rare, however, on plants outside the cultivated area. It multiplied readily on sugar beet and mangel-wurzel and was able to breed on *Amaranthus* spp., *D. stramonium*, *D. meteloides*, *Z. elegans*, and to a limited extent on *C. album*. It failed to breed on tomato, Turkish tobacco, and *Nicotiana glutinosa*.

Agalliana ensigera feeds on the phloem of sugar beet and leaves a partial sheath of salivary secretion along the line of puncture. It is able to pick up virus from liquids containing phloem exudate of diseased beets but, unlike *Eutettix tenellus*, the vector of North American curly top virus, it was not observed to leave salivary deposits in the liquid media on which it fed. The minimum incubation period of the virus in the insect, as found, was 24 to 72 hours. Virus in viruliferous insects was not exhausted by a 36-day feeding period on a plant immune to curly top. Limited tests indicate that the virus does not pass through the egg stage of the insect.

The virus of Argentine curly top appears to occur in relatively high concentration in the phloem of infected sugar beets and mangel-wurzels. The thermal inactivation point seems to be between 75° and 80° C. The virus was not inactivated by a short exposure to 50-percent alcohol. By means of phloem exudate from infected sugar beets, the virus was transmitted to 4 of 20 rapidly growing sugar beets by needle inoculation, but no infection resulted from rubbing inoculum over the surface of leaves. The virus moved from the point of introduction by leafhopper inoculation at the distal end of a beet leaf downward through the leaf, a distance of 15 cm., in 2 hours. These properties of Argentine curly top virus parallel those of North American curly top virus, so far as known.

The Argentine curly top virus differs from curly top virus in North America in vector relations and degree of recovery of affected plants as the disease progresses. It resembles curly top virus of North America in the general type of symptoms on known host plants, in host range, so far as is known, except for members of the family Solanaceae, in known properties, and in the resistance manifested by tested sugar-beet varieties. These similarities appear so significant that, despite the differences in vector relations and certain known symptomological and host differences, it seems best to classify, tentatively at least, the

Argentine curly top virus as a variety of the curly top virus complex (*Ruga verrucosans* C. and B.) of North America. The variety name *distans* is suggested for the Argentine virus.

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RELATION OF TEMPERATURE TO REPRODUCTION IN SUGAR BEETS¹

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INTRODUCTION

The influence of temperature on sexual reproduction in the sugar beet (*Beta vulgaris* L.) has long been considered a subject of importance. Shaw (14, p. 112),² strongly influenced by some of the work of Klebs on reproduction in plants, studied the problem extensively and concluded that "the range of temperature within which reproductivity is determined evidently lies between 2.75° C. and 10° C., or very near those limits." Shaw showed that at temperatures below 1.75° the beets remained dormant, and he believed that this dormancy, imposed by low temperature, was responsible for the failure of such beets to develop reproductively. He reasoned that some areas were unfavorable for the reproductive development of beets because silo temperatures were too cool during the winter and because the transition period in the spring, between the prevalence of temperatures too low to allow planting and the incidence of unfavorably high temperatures, was too short to afford an adequate period of "restrained growth." Shaw based his conclusion that 10° is near the upper limit at which reproductivity is determined on a comparison of winter temperature records and his observation of the fact that only part of the plants produced seedstalks in fields of beets planted during November in the Salt River Valley of Arizona.

Shaw pointed out that the mean winter temperature of the natural habitat of the wild beet (*Beta maritima* L.) along the northern coast of the Adriatic Sea insured reproductivity in beets. A mean temperature curve for that area was composited from the mean temperatures of Venice, Trieste, Fiume, Pola, and Zara. By a composite curve of mean temperatures for the coastal area of Oregon and Washington, derived by averaging the mean temperatures of Portland and Roseburg, Oreg., and Seattle, Wash., Shaw showed that the winters of that area are similar to those in the natural habitat of the wild beet. The curves compared by Shaw indicated that the mean temperature range that he considered favorable to "reproductive determination" extended over an even longer period in the coastal area of Oregon and Washington than on the northern coast of the Adriatic.

Pack (13) found that the most favorable storage temperature for inducing reproductive development in sugar beets was near 4.4° C., but he did not confirm Shaw's conclusion that storage at temperatures near 0° induced dormancy and thus failed to induce reproductive

¹ Received for publication May 10, 1944.

² Italic numbers in parentheses refer to Literature Cited, p. 68.

growth. Pack (12) also found that sugar beets which had been stored under optimum temperature conditions (4°) for seed production could be forced into the various degrees of reproductiveness, ranging from true seed types to almost completely vegetative types, by controlling the time of year that mother beets were planted out. Early planting gave the best reproductive development. The fact that in the late plantings some of the beets developed vegetatively suggests that the high temperatures of late spring and early summer caused a reversal of the reproductive process.

Steinberg and Garner (15) found that without any cool-temperature exposure a selected strain of sugar beet³ could be induced to flower at 73° F. by the use of continuous high-intensity illumination. Flowering did not occur at 73° when the plants were exposed to daily illumination periods of 18 hours, but it did occur at 60° and at 65° under 18-hour photoperiods. With continuous illumination, the period from germination to flowering decreased as the temperature was raised, being 65 days at 60°, 48 days at 65°, and 39 days at 73°.

Owen et al. (11) showed that in a sugar-beet variety of extremely low bolting tendency storage for 3 months at 33° to 36° F. did not induce bolting except when the plants were transplanted to an environment favorable for reproduction (affording long photoperiods and cool temperatures). A longer period of storage (172 days) of beets of the same variety and at the same temperature resulted in bolting in all the plants, even in an environment unfavorable for bolting (affording short photoperiods and warm temperatures). Evidently a high degree or large amount of thermal induction had been finally effected at the cool temperature by prolonging the storage period.

Apparently either photoperiodic induction or thermal induction, acting independently, can carry the reproductive process far toward completion. The effects of light and temperature on reproduction in the sugar beet seem to be complementary.

The studies reported in this paper deal principally with the relation of temperature to reproductivity in the sugar beet.

MATERIALS AND METHODS

Storage experiments were conducted with eight curly-top-resistant varieties of sugar beets. These varieties, U. S.⁴ 15, U. S. 22, S. L.⁴ 68, S. L. 234, S. L. 235, S. L. 979, S. L. 1-11, and S. L. 1-121, were known to differ widely in bolting tendency. U. S. 15, S. L. 234, and S. L. 979 require a relatively long cool-temperature treatment before they can be induced to form seedstalks and are, therefore, termed nonbolting varieties. U. S. 22, S. L. 235, S. L. 1-11, and S. L. 1-121 are intermediate in bolting tendency. S. L. 68 requires a relatively short period of cool-temperature exposure to induce bolting and is therefore usually referred to as an easy-bolting variety.

Cool-temperature exposure induces changes that result in subsequent reproductive development when the sugar beet is grown under long daily photoperiods. This process is referred to herein as thermal induction and is measured by the relative rate and percentage of plants bolting. Warm-temperature exposure, following adequate thermal induction, was found to reduce or neutralize the effect of

³ The seed, supplied by G. H. Coons, Division of Sugar Plant Investigations, was from the easy-bolting variety 00569-0.

⁴ Varieties designated "U. S." are commercial varieties grown in the western part of the United States. Those designated "S. L." are varieties bred at the U. S. Sugar Plant Field Laboratory, Salt Lake City, Utah.

cool-temperature exposure and is referred to as reversal of thermal induction.

"Bolting," as used in this paper, refers to the appearance of a seed-stalk, whether or not subsequent flowering occurs (10, 11, 16).

To avoid misunderstandings and confusion regarding expressions relating to temperature ranges, the writer proposes to use certain arbitrarily chosen terms with meanings as follows: Low, near or below freezing; cool, above freezing to about 15° C.; warm, about 15° to 30°; and high, above 30°.

Sugar beets were stored at a series of temperatures, beginning at the lowest temperature that could be used without injury to the roots and increasing, by 2° to 3° C., up to the maximum temperature at which thermal induction of reproductive development occurred. Lack of adequate facilities limited the range of the experimental storage temperatures, especially between 10° and 15°. Fairly close control was maintained on temperatures at or slightly below 0°, but temperatures above about 1.5°, in the commercial cold-storage rooms used, were less subject to control. Maximum- and minimum-registering thermometers placed with each lot of beets usually showed a variation of less than 0.7° in lots stored at temperatures lower than 1.5°, whereas the temperature of other lots varied as much as 2°. For this reason the average maximum and minimum temperatures are usually given as the range for each stored lot. In one of the later experiments on reversal of thermal induction some of the sugar beets were stored in electrically heated incubator ovens that were placed in a cool room. This was found to be a more troublesome method of storage owing to the condensation of moisture on the inside of the ovens. The average temperatures recorded in these later experiments are the means of twice-daily readings.

In most experiments the sugar beets were taken from the field in midsummer to avoid the thermal induction of reproductive development that occurs naturally with fall temperatures. A comparison was also made between roots stored at various known temperatures and those that had remained in the field near Harrisburg, Oreg., during most of the winter.⁵ The sugar beets that were used in experiments to determine the reversibility of thermal induction were taken from overwintering fields after sufficient cool-temperature exposure to cause most plants to bolt when subsequently replanted in a favorable photoperiodic environment.

All lots of sugar beets were taken from moist soil, and the leaves were carefully trimmed off without injury to the central crown bud. They were then placed in large, clean, galvanized-iron cans and taken to storage. Drying of roots during storage was avoided by keeping a small amount of water in the cans. False bottoms of perforated metal, 1 to 2 inches high, were placed in each can to allow air circulation within the can and to prevent the beets from coming in direct contact with water. At storage temperatures above 15° C., wet excelsior, placed both above and below the roots, was used in some instances, and such lots were removed frequently for examination and to aerate the cans.

Roots held at low temperatures were allowed to warm up slowly when taken from storage. The leaf growth on beets stored at warmer

⁵ Sugar beets received from Harrisburg, Oreg., were shipped to the writer through the courtesy of the West Coast Beet Seed Co.

temperatures was trimmed off, and the roots were replanted in a greenhouse floor bed or in the field. Greenhouse temperatures were kept relatively warm, and field plantings were delayed until late spring to avoid further thermal induction after replanting.

Since the effects of long daily photoperiods and prolonged cool-temperature exposure are apparently complementary in inducing reproductive development in biennial beets, long photoperiods (either 17 to 18 or 24 hours daily) were chosen so as to bring out the differential bolting response to thermal treatments. During tests made in the greenhouse in the winter and early spring, artificial illumination (25 to 50 foot-candles) supplementary to daylight was supplied by Mazda lamps equipped with reflectors.

EXPERIMENTAL RESULTS

THERMAL INDUCTION IN SUGAR BEETS TAKEN FROM THE FIELD DURING THE SUMMER

Six lots of sugar beets of the easy-bolting variety S. L. 68 were stored at different temperatures from July 22 to September 13, 1939. All lots were replanted in a warm greenhouse September 13, and were

TABLE 1.—*Effect of various storage temperatures on thermal induction in sugar beets as shown by bolting response of different varieties replanted in a warm greenhouse under continuous illumination or 17- to 18-hour photoperiods*

Variety, storage period, photoperiod, and treatment designation	Storage temperature		Beets observed	Beets bolting at end of indicated period (days) after being replanted													
	Average minimum	Average maximum															
				17	24	25	28	31	34	40	45	47	50	53	56		
S. L. 68 (July 22-Sept. 13, 1939); continuous illumination:	° C.	° C.	No.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	
A	-0.8	0.7	30	0	0			3.3			56.7						
B	1.8	2.7	30	0	3.3			3.3			63.3						
C	1.3	3.2	30	0	3.3			10.0			70.0						
D	2.7	3.4	30	0	16.7			53.3			90.0						
E	-6.2	7.6	30	23.3	80.0			100.0			100.0						
F	9.5	10.2	30	38.0	82.9			93.1			100.0						
S. L. 68 ¹ (Sept. 6-Oct. 25, 1940); 17- to 18-hour photoperiods:																	
A	-1.0	-5	50		0			0		0					10.0		
B	0	1.7	50		0			0		10.0					26.0		
C	4.0	6.9	50		18.0			52.0		90.0					100.0		
D	5.5	6.7	50		34.0			72.0		92.0					100.0		
U. S. 15 (July 22-Oct. 18, 1939 ²); continuous illumination:																	
A	-1.1	.4	30				0		0	6.7					10.0		
B	.6	2.1	30				0		0	3.3					13.3		
C	1.4	2.3	30				0		0	0					20.0		
D	2.4	3.1	30				0		3.3	10.0					16.7		
E	4.7	6.3	30				30.0		60.0	70.0					86.7		
F	8.4	9.7	30				23.3		33.3	43.4					53.3		
U. S. 22 ³ (Nov. 27, 1940-Jan. 16, 1941); 17- to 18-hour photoperiods:																	
A	-1.3	-8	35			0								5.7		5.7	
B	.8	2.0	40			0								17.5		20.0	
C	4.2	5.3	40			7.5								67.5		80.0	
D	4.4	5.8	40			22.5								72.5		87.5	

¹ The beets were taken from the field Aug. 28 and held at 0.6° to 1.7° C. until Sept. 6, 1940, when they were divided into 4 lots and stored at different temperatures.

² Storage temperatures were the same as those for S. L. 68 for first 53 days; from Sept. 14 to Oct. 18, lower outside temperatures affected storage temperatures enough to lower nearly all the general averages.

³ The beets were taken from the field Aug. 28 and held at approximately 0.5° to 1.0° C. for 1 month, then at -1.0° to -0.5° until Nov. 27, 1940, when they were divided into 4 lots and stored at different temperatures.

continuously illuminated thereafter. Data in table 1 and figure 1 indicate that thermal induction proceeded at a relatively slow rate at -0.8° to 0.7° C., and that the maximum rate was at temperatures above 6° and probably near 10° . The data also indicate that even at the lowest temperature, where the rate of thermal induction was



FIGURE 1.—Effect of different storage temperatures on thermal induction in sugar beets as shown by the bolting response of variety S. L. 68 replanted in a warm greenhouse under continuous illumination after 53 days of storage. Average minimum and maximum storage temperatures of respective treatments: A, -0.8° to 0.7° C.; B, 1.8° to 2.7° ; C, 1.3° to 3.2° ; D, 2.7° to 3.4° ; E, 6.2° to 7.6° ; F, 9.5° to 10.2° . Photographed November 8, 1939, 56 days after being replanted.

relatively slow, the process went far enough to result, after only 53 days of storage, in a large percentage of plants bolting.

In another test with variety S. L. 68, the roots were taken from the field August 28, 1940, and held at 0.6° to 1.7° C. until September 6. On the latter date they were divided into four lots and stored at different temperatures until October 25, when they were replanted in

the greenhouse under 17- to 18-hour photoperiods. The data in table 1 and figure 2 show that there was very little thermal induction at -1.0° to -0.5° and that there was an increase in the rate of the process at each increase in storage temperature. As in the previous experiment with this variety, a relatively short storage period, even at the lowest temperature, afforded sufficient thermal induction to result in bolting in some plants.

The photographs in figure 2 show even greater differences in reproductive growth than are indicated by the data in table 1. Most of the sugar beets previously stored at the higher temperatures were flowering, whereas only a few seedstalks had been initiated in the beets stored at temperatures below 0° C., and most of these seedstalks were semivegetative in appearance.

Sugar beets of the nonbolting variety U. S. 15 were stored from July 22 to October 18, 1939, at approximately the same range of temperatures used that season with variety S. L. 68 (table 1). Because of lower outside temperatures, the average minimum and maximum temperatures in this test (table 1) were nearly all cooler than the corresponding temperatures in the former experiment.

Decayed spots developed in the crowns of some of the sugar beets of variety U. S. 15 stored at the two highest temperatures (table 1, treatments E and F), necessitating rather severe trimming of the crowns before the beets were replanted. It was thought that this might reduce bolting, but it is now believed that such is not the case. Although the tallest seedstalks were among those that developed from the lot stored at 8.4° to 9.7° C. (treatment F), the most rapid rate and the highest percentage of plants bolting occurred in the lot stored at 4.7° to 6.3° (treatment E).

The rate of thermal induction in U. S. 15, as in S. L. 68, was slower at the lower storage temperatures, as indicated by the slower bolting rate and smaller percentage of plants bolting. Unlike S. L. 68, however, U. S. 15 did not receive enough thermal induction for complete bolting at the storage temperature previously found to be the most favorable, even though the storage period was considerably longer than in the tests with S. L. 68. Obviously, U. S. 15 requires more extensive thermal induction. Another point believed to be significant is that apparently a storage temperature of 8.4° to 9.7° C. was higher than optimum for thermal induction in U. S. 15. Perhaps a much longer storage period at a relatively cool temperature would be necessary for adequate thermal induction in this variety.

A storage experiment with variety U. S. 22 was started August 28, 1940. The roots were taken from the field and held for 31 days at approximately 0.5° to 1.0° C.; then they were moved to a storage chamber in which the temperature ranged from -1.0° to -0.5° . On November 27, the beets were divided into four lots, which were stored respectively, at the temperatures shown in table 1. The temperature in the coldest chamber was reduced to about -2.0° for the first 2 weeks, when it was observed that nearly all the roots were injured superficially by freezing. However, after the beets were transplanted, only one of the plants failed to grow, and four others died during the experiment. It seems probable that the very limited amount of thermal induction in this case occurred during the 31 days of preliminary storage, when the temperature was at all times slightly above

0°, and that during the 110 days when the temperature was continuously below 0° the sugar beets were so dormant that thermal in-

FIGURE 2. Effect of different storage temperatures on thermal induction in sugar beets as shown by the bolting response of variety S. L. 68, replanted in a warm room under 17- to 18-hour photoperiods after 49 days of storage. Average minimum and maximum storage temperatures of respective treatments: A, -1.0° to -0.5° C.; B, 0° to 1.7°; C, 4.0° to 6.9°; D, 5.5° to 6.7°. Photographed January 15, 1941, 82 days after being replanted.



duction was practically arrested. However, when beets that had been kept virtually dormant at near 0° were stored at approximately 5° for 50 days, a high percentage of bolting resulted, indicating that thermal

induction at the higher temperature was fairly rapid. The data also indicate that at similar temperatures U. S. 22 requires a longer storage period than does S. L. 68 to induce bolting in an equal percentage of plants.

THERMAL INDUCTION IN SUGAR BEETS TAKEN FROM THE FIELD DURING THE WINTER

Sugar beets taken from the field in the summer and those taken from the field in winter were compared to determine the effect of cool-temperature storage. The results reported in table 1 were obtained with roots taken from the field before the occurrence of temperatures cool enough for thermal induction. In these tests, therefore, only the thermal effects during storage were involved. In contrast to tests with summer-harvested roots, an experiment was conducted in which sugar beets of the nonbolting variety S. L. 979 that had grown in the field at St. George, Utah, from September 16 to December 6, 1939, were brought to Salt Lake City and stored at various temperatures for 44 days. They were then replanted in the greenhouse under continuous illumination. One lot of plants of the same variety, which had grown in the field at Salt Lake City from August 16, 1939, to January 19, 1940, was transplanted to the greenhouse with the stored lots. The treatments used and results obtained are given in table 2.

TABLE 2.—*Effect of different storage temperatures on thermal induction in sugar beets as shown by bolting response of variety S. L. 979 replanted in a warm greenhouse under continuous illumination*

[Storage period 44 days, Dec. 6, 1939, to Jan. 19, 1940]

Treatment ¹	Storage temperature		Beets ² bolting at end of indicated period (days) after being replanted		
	Average minimum	Average maximum	26	36	52
	° C.	° C.	Percent	Percent	Percent
A.....	0.2	1.3	9.3	37.2	51.2
B.....	1.6	2.3	11.6	44.2	65.2
C.....	4.7	6.7	32.6	55.8	69.7
D.....	6.1	7.1	32.6	58.2	74.5
E.....	6.5	8.2	34.9	62.8	72.1
F.....			67.5	90.7	95.4

¹ Beets receiving treatments A to E were grown at St. George, Utah; those receiving treatment F were grown near Salt Lake City, Utah, and transplanted directly from the field.

² Percentages are based on a total of 43 beets observed for each treatment.

The sugar beets taken from the field in early December and stored at 0.2° to 1.3° C. for 44 days bolted at a faster rate and in higher percentages than did U. S. 15 beets of comparable inherent bolting tendency taken from the field in July and stored for 88 days at approximately the same temperature (treatment A in table 1). The sugar beets that were transplanted directly from the field at Salt Lake City to the greenhouse in January bolted even more rapidly and in greater numbers than the sugar beets from St. George that had been stored. Obviously, some thermal induction of reproductive development had occurred in both lots during the time they were exposed to fall and early-winter temperatures in the field. On the other hand, as shown by previous experiments, in the beets taken from the field in July, bolting was thermally induced only during storage.

Another comparison of the effect of thermal exposure of sugar beets in the field with that of thermal exposure during storage was made in a field test planting in 1940. Mother beets of the nonbolting variety U. S. 15 were harvested near Salt Lake City during October 1939 and stored at two different temperatures. Steckling beets of the same variety were taken from an overwintering beet-seed field near Harrisburg, Oreg., March 10, 1940. The three lots were replanted in rich garden soil at Heber City, Utah, April 5, 1940. Photographs taken 77 days after the beets were replanted (fig. 3) show that the sugar beets that had overwintered in the field (fig. 3, *C*) were beginning to flower before seedstalks were produced on the lot stored at 0° to 1.7° C. (fig. 3, *A*). The beets stored at an average temperature of 4.4° (fig. 3, *B*) were intermediate in development between the two extremes. The more advanced stage of reproductive development of the steckling beets indicates that thermal induction may be more completely accomplished by overwintering sugar beets under optimum conditions in the field than by storing them at favorable temperatures.

REVERSIBILITY OF THERMAL INDUCTION

There has been some difference of opinion regarding the reversibility of developmental processes in plants. According to the Imperial Bureau of Plant Genetics (5), Lysenko believed that these processes take place in definite steps and sequence, that a qualitative change in the protoplasm induces morphological changes, and that the steps that must take place in the plant before it reaches sexual maturity are irreversible. Ljubimenko and other Russian workers (see 5) believed that the presence of a developmental hormone is responsible for the morphological changes involved in reproductive development and that the developmental processes are reversible. Many instances have been reported showing that the type of development may be reversed. Chroboczek (3) reversed the development of red garden beets several times in the same plant by changing the thermal and photoperiodic environment. This method was also used by Owen (9) to produce semivegetative seedstalks in sugar beets for use as cuttings.

Since thermal induction in sugar beets occurs only at relatively cool temperatures, it seems probable that substances conducive to bolting are formed in beets stored at those temperatures and that at warmer temperatures these substances are not formed or at least not accumulated. The rate of thermal induction, within the favorable temperature range, increases as the temperature rises. It is probable, therefore, that biochemical processes that are accelerated by an increase in temperature are involved. In view of this, if relatively warm storage temperatures were found to result in reversal of thermal induction, it is reasonable to expect that the rate of reversal would be relatively rapid. Any observed reversal could be attributed to thermal alteration of the biochemical equilibrium of the plant, resulting in a change in the direction of the biochemical processes involved. This line of thought led to experiments in which sugar beets that had received some thermal induction were stored at an intermediate range of temperatures (9° to 25° C.) for short periods to determine whether reversal could be so induced.

The sugar beets used in one such test were of the variety S. L. 1-121, classed as intermediate in bolting tendency. They were

taken from an overwintering seed field near St. George, Utah, about January 20, 1942. One lot was directly replanted under continuous



FIGURE 3.—Comparison of effects of different storage temperatures and of overwintering in the field on thermal induction in sugar beets as shown by the bolting response of variety U. S. 15, replanted in the field at Heber City, Utah, April 5, 1940: A, Mother beets stored at 0° to 1.7° C.; B, mother beets stored at an average of 4.4°; C, steckling beets taken from an overwintering beet-seed field near Harrisburg, Oreg., March 10, 1940. Photographed June 21, 1940.

illumination in a warm greenhouse room; another lot was stored at 17° to 18° C., and a third lot was stored at 23° to 24°. Plantings from each stored lot were made at semiweekly intervals.

The data in table 3 show that there was some reduction in the rate of bolting of plants that were stored at 17° to 18° C. for a period of about 10 days. All the beets stored at this temperature, however, had bolted 51 days after being replanted. There was a much greater reduction of bolting in beets that were stored at 23° to 24°. Of the beets stored at this temperature for 21 days, only 20 percent bolted in 51 days.

TABLE 3.—*Effect of storage at warm temperatures on the reversal of thermal induction in sugar beets as shown by the bolting response of variety S. L. 1-121 when replanted in a warm greenhouse under continuous illumination*

Storage temperature and period	Beets observed	Beets bolting at end of indicated period (days) after being replanted				
		23	30	37	44	51
		Percent	Percent	Percent	Percent	Percent
Check (not stored).....	20	30	90	100	100	100
17° to 18° C.:						
3 days.....	20	20	75	95	100	100
7 days.....	20	5	75	95	100	100
10 days.....	10	0	40	100	100	100
13 days.....	10	10	80	90	90	100
23° to 24° C.:						
3 days.....	20	0	30	90	100	100
7 days.....	20	0	50	85	90	90
10 days.....	20	0	55	75	75	75
13 days.....	20	0	40	60	80	85
17 days.....	20	0	5	35	45	50
21 days.....	20	0	5	15	20	20

Sugar beets of the intermediate-bolting variety S. L. 1-11 were obtained from overwintering fields in St. George, Utah, and Talent, Oreg., about January 20, 1942. They were stored at -0.5° C. until April 20, when a sample from each lot was moved to room temperature (average 25.6°) for a period of 16 days before all lots were replanted in the field May 6, 1942. Of the beets given the warm-temperature treatment only 1 percent bolted, whereas 92.6 percent of the others bolted (table 4 and fig. 4).

TABLE 4.—*Reversal of thermal induction in sugar-beet variety S. L. 1-11 by a short period of storage at a relatively warm temperature*

Treatment	Average storage temperature		Beets observed	Beets bolting	
	Jan. 20 to Apr. 20	Apr. 20 to May 6		June 16	July 22
	° C.	° C.	Number	Percent	Percent
A.....	-0.5	-0.5	1,699	87.3	92.6
B.....	-5	25.6	395	0	1.0

To test further the lower limits of temperatures that result in reversal of thermal induction and to obtain information regarding the rate at which these changes are effected, another test was made in 1943. On March 4, plants of three sugar-beet varieties—U. S. 22 and S. L. 235, which are intermediate in bolting tendency, and S. L. 234, a nonbolting variety—were taken from an overwintering seed field planted in St. George, Utah, September 7, 1942. The beets

of each variety were divided into nine lots. All were stored uniformly at a low temperature until March 24, when five lots of each variety were moved to warmer temperatures. Details of these storage

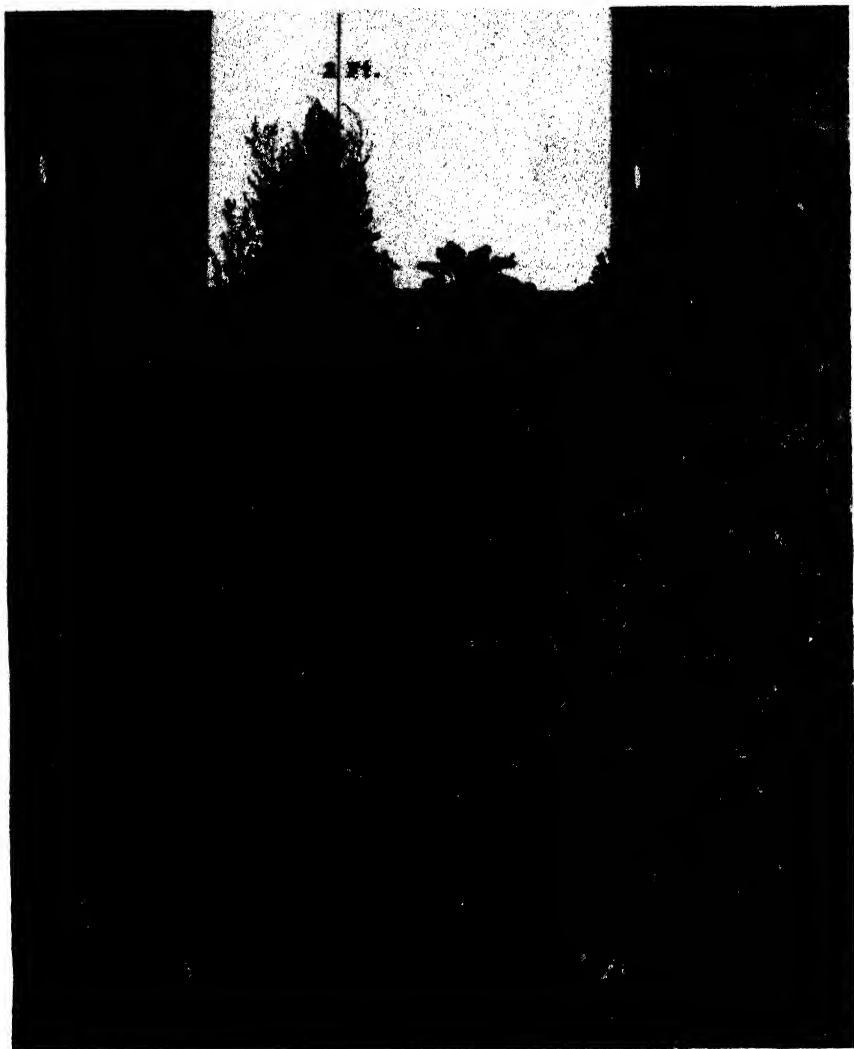


FIGURE 4.—Reversal of thermal induction at a relatively warm temperature. The sugar beets in rows *A* and *B* were taken from an overwintering seed field in January 1942 and stored at -0.5° C. until April 20, when those in row *B* were removed from the low-temperature chamber and stored at approximately 26° . Sixteen days later both lots were replanted in the field. Photographed June 28, 1942.

temperatures are shown in table 5. The other four lots were held at -0.85° C. until April 12, when two more lots from each variety were moved to warm temperatures. The two remaining lots of each variety were kept at -0.85° as checks. All lots of beets were replanted in the field April 29, 1943. Those beets that were seriously injured

or severely affected by curly top were eliminated before bolting counts were made.

Some difficulty was encountered in storing the sugar beets at 16.4° C. for 36 days. Electrically heated incubator ovens placed in a cool room were used as temperature chambers. The beets were placed on trays and water was kept in pans inside the ovens to maintain high humidity. The thermal differential between the inside of the ovens and the outside air caused moisture to condense on the inside and drip on some of the beets. This difficulty resulted in considerable spoilage in these lots. Although the percentage of plants bolting in this lot is based on a small number, the results are in line with the data based on larger numbers in lots in which there was little injury from spoilage.

TABLE 5.—*Effect of storage periods, differing in temperature and duration, on reversal of thermal induction in sugar beets as shown by bolting response of varieties U. S. 22, S. L. 234, and S. L. 235, when replanted in the field April 29, 1943*

Variety	Storage period		Beets observed	Beets bolting at end of indicated period (days) after being replanted		
	Temperature	Duration		30	43	50
	° C.	Days	Number	Percent	Percent	Percent
U. S. 22	-0.85	36	114	96.5	100.0	100.0
	9.1	36	56	94.6	98.2	100.0
	10.6	36	45	93.3	100.0	100.0
	13.2	36	25	12.0	48.0	56.0
	16.4	36	4	0	0	0
	19.3	17	51	19.6	49.1	51.0
	23.8	17	49	0	4.1	8.2
	(1)	36	34	14.7	20.6	20.6
	- .85	36	83	84.4	96.3	98.8
	9.1	36	51	74.5	98.0	98.0
S. L. 234	10.6	36	39	41.1	79.5	79.5
	13.2	36	17	0	23.5	35.3
	16.4	36	4	0	0	0
	19.3	17	16	6.2	12.5	12.5
	23.8	17	23	0	0	0
	(1)	36	34	0	14.7	20.6
	- .85	36	99	87.9	100.0	100.0
S. L. 235	9.1	36	49	95.9	100.0	100.0
	10.6	36	35	82.9	91.5	97.2
	13.2	36	13	53.8	53.8	69.2
	16.4	36	8	0	0	0
	19.3	17	45	31.1	60.0	62.2
	23.8	17	47	0	2.1	6.4
	(1)	36	29	10.3	24.1	27.6

¹ Beets were stored at average alternating temperatures of 9.1° C. for 16 hours and approximately 26° for hours daily for 36 days before they were replanted.

The data in table 5 show that some reversal of thermal induction occurred at temperatures near 10° to 12° C. About the same amount of reversal was effected in sugar beets stored at 13.2° for 36 days as in similar beets stored at 19.3° for only 17 days. This indicates that the rate of reversal at 19.3° was approximately twice as great as at 13.2°. There was a rather striking reduction in the percentage of beets bolting in the nonbolting variety S. L. 234 stored at 10.6°. In the two other varieties the reduction in plants bolting after storage at this temperature was so small as to be of questionable significance. At 13.2° all the varieties showed pronounced reduction in percentage of plants bolting.

Those sugar beets stored at alternating temperatures for the entire period of 36 days showed a marked reduction in bolting response.

There was evidently greater reversal of thermal induction in the beets stored at these daily alternating temperatures for 36 days than in those stored continuously at 19.3° C. for 17 days, but less than in those

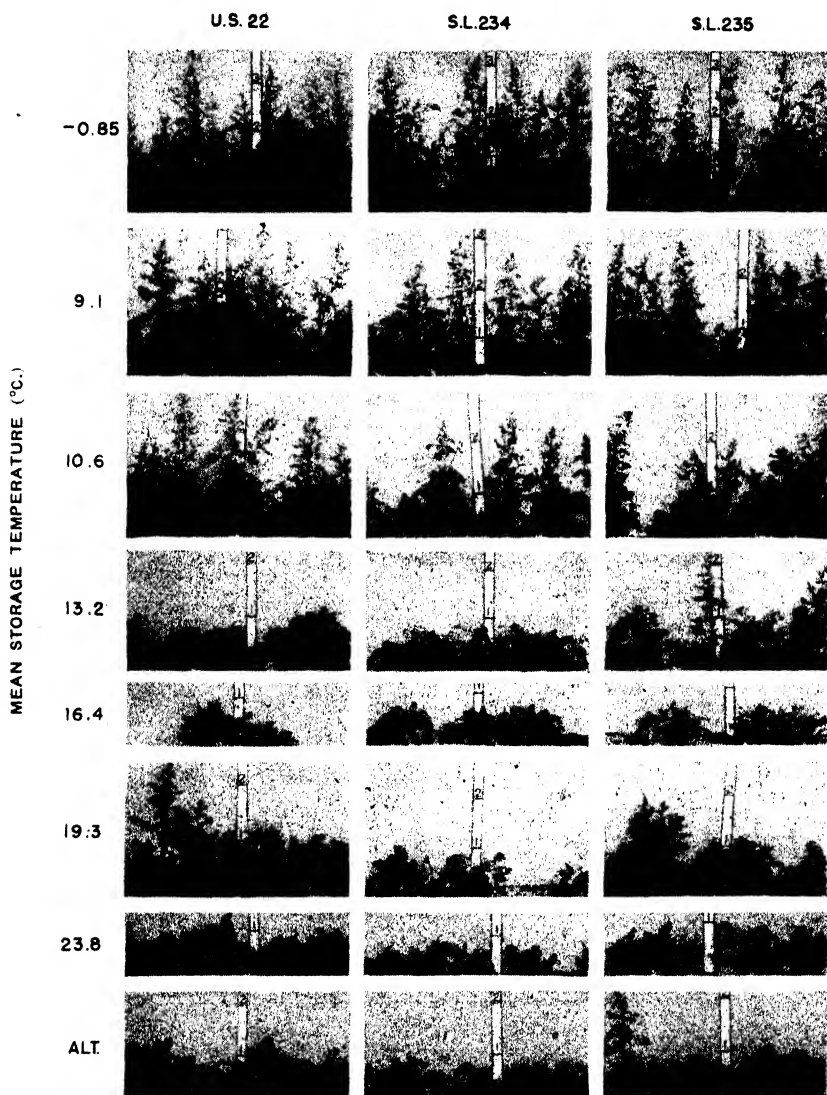


FIGURE 5.—Representative beets from treatments listed in table 5. Storage periods as follows: -0.85° C., for 36 days; 9.1°, for 36 days; 10.6°, for 36 days; 13.2°, for 36 days; 16.4°, for 36 days; 19.3°, for 17 days; 23.8°, for 17 days; daily alternating (Alt.) temperatures, for 36 days. Photographed June 22, 1943, 54 days after being replanted. (Small numbers in each photograph indicate height in feet.)

stored continuously at 23.8° for 17 days. A composite photograph of representative sugar beets in each plot, taken 54 days after being replanted, is shown in figure 5.

DISCUSSION

The existence of a substance or substances that may be responsible for reproductive development in higher plants has been suggested by many writers (2, 6, 7, 8). The principal basis for this assumption has been the demonstration that photoperiodically produced substances or stimuli (1, 4) may be transferred from one part of a plant to another through grafts or by manipulating light exposure or foliar growth. Sugar beets apparently develop reproductively as a result of the production and accumulation of a substance or substances in sufficient quantities to cause the morphological changes that occur. The influence of temperature on the rate and reversibility of the process seems to support the assumption that it is of a chemical nature. The data in table 5 show that the rate of reversal was approximately twice as fast at 19.3° C. as at 13.2°. This indicates a temperature coefficient of approximately 3.3 for the rate of reversal between 13.2° and 19.3°. The fact that the temperature coefficient of reversal is high, while not considered as proving the chemical nature of the process, is thought to afford evidence supporting this viewpoint, inasmuch as the usually accepted value of the temperature coefficient of purely physical processes is relatively low, ranging from 1.2 to 1.4 at the temperatures that ordinarily prevail in the plant.

The available data on thermal induction and on the reversal of the process in sugar beets seem to justify the assumption that this induction process may be considered to exist in a state of complete reversibility until sufficient seedstalk development occurs to partially prevent reversal of reproductive development. That this morphological differentiation exerts a restraining influence on reversal is shown by the fact that reproductive development usually continues in spite of the high temperatures that prevail in some seed-growing areas during the period of rapid seedstalk growth and flowering.

A gradient in the amount of thermal induction between the small amount occurring at a relatively warm temperature and the large amount attainable at a cool temperature is indicated by several lines of evidence. At warmer temperatures a little thermal induction will occur if the beets involved have not been thermally induced previously. If the beets involved have already received extensive thermal induction, holding them at the warmer temperature will result in reversal of thermal induction toward the equilibrium level for that temperature.

An example of one line of evidence of the gradient in extent or amount of thermal induction has been reported previously (11). Clone No. 138 is a nonbolting type and remains vegetative under conditions which cause most sugar beets to develop seedstalks. An 87-day treatment of plants of this clone at 0.5° to 2.2° C. did not induce bolting except when followed by a favorable environment for bolting, that is, one affording a long photoperiod and moderately cool temperature. However, storage for 172 days at 0.5° to 2.2° induced bolting even in an environment that was unfavorable, that is, one affording short photoperiods and relatively warm temperatures. This indicates that there was more extensive thermal induction as a result of the longer period of storage at cool temperatures. Experiments with other varieties gave similar results.

Another line of evidence is afforded by comparing the responses of the varieties S. L. 68 and U. S. 15. The former variety is an easy-

bolting type, whereas the latter is decidedly nonbolting. Plants of S. L. 68, stored 53 days at 9.5° to 10.2° C., received enough thermal induction to effect complete bolting under very favorable photoperiodic conditions. U. S. 15, on the other hand, bolted incompletely under a similar photoperiodic environment after storage for 88 days, even at 4.7° to 6.3°, a temperature evidently more favorable for thermal induction (table 1) than the higher temperature found to be adequate with S. L. 68. There was more bolting in beets of variety U. S. 15 stored at 4.7° to 6.3° than in those stored at 8.4° to 9.7°. From these facts it appears that the temperature limitation in the relatively warmer ranges permits an amount of thermal induction that is adequate for complete bolting in S. L. 68, but inadequate in the case of U. S. 15.

The bolting responses of the nonbolting variety S. L. 234 and the two intermediate-bolting varieties U. S. 22 and S. L. 235 afford further pertinent evidence. The data in table 5 and figure 5 show that there was an appreciable reduction in bolting of S. L. 234 after storage at 10.6° C. for 36 days; U. S. 22 and S. L. 235 showed little reduction in bolting after storage at 10.6°, but a definite reduction in bolting after storage at 13.2°.

The bolting response of a hypothetical population of beets representing all possible degrees of bolting tendency from easy-bolting to the most nonbolting types and grown at temperatures ranging from 3° to 18° C. for a long period before they are subjected to more rapid growing temperatures and long photoperiods, might be represented by a curve similar to *EF* in figure 6. That is, only the easy-bolting types would bolt after exposure at 12° to 15°; others, of intermediate-bolting tendency, would bolt after exposure at 8° to 10°; and still more nonbolting types might bolt after exposure to cooler temperatures, depending upon the amount or degree of induction necessary to satisfy the thermal-induction requirement of each type. The above-mentioned data seem to indicate that essentially the same result might be approached, either by thermal induction of beets not thermally induced or by reversal of thermally induced ones.

The foregoing facts and inferences seem to support the hypothesis that a high degree of thermal induction is attainable at a comparatively cool temperature, that a low degree is possible at the other extreme of the temperature range within which the process can take place, and that for each degree of temperature between these points there is a corresponding, intermediate degree of thermal induction attainable. According to this view there is a gradient in the degree of thermal induction, dependent on temperature.

The direction of the thermally induced changes (thermal induction or reversal) is thus dependent upon the storage temperature and the amount or degree of thermal induction attained before storage. Thermal induction seems always to increase, although slowly, at temperatures near the freezing point. The amount of thermal induction attainable as temperatures are increased, decreases until, at 12° to 15° C., little or none occurs. At temperatures above about 15° any appreciable amount of thermal induction effected previously is reversed. If a large amount of thermal induction has been effected before experimental storage, a partial reversal will occur if the storage temperature, even though cool, is higher than that to which the beets were previously exposed. On the other hand, if no thermal

induction has been effected previously, an observable amount of thermal induction may be effected in an easy-bolting variety at temperatures even as high as 12° to 15°. Thus there is an intermediate range of temperatures at which either thermal induction or reversal may occur. This is believed to indicate a shift in the biochemical equilibrium of the plant as a result of change in temperature. Since the process is thermally reversible, it might be represented by an equation in which Ro represents a condition favoring reproductive development while ro represents a condition favoring vegetative development ($Ro \rightleftharpoons ro$). A graphic illustration of this concept is shown by the curve EF , drawn in relation to the left and base axes of figure 6. It should be borne in mind that the curve illustrates the ultimate amount or degree of thermal induction that might result at a given temperature regardless of the direction of approach or the time required to establish equilibrium.

Temperature also influences the *rate* of thermal induction and of reversal. The fact that the rate of thermal induction is greatly reduced as storage temperatures approach the freezing point indicates that this process is retarded as the biochemical processes involved approach quiescence. Within the favorable temperature range for thermal induction, the rate increases as the temperature rises. The fact that the rate of reversal similarly increases as the temperature rises indicates that, even though the directions of these changes in developmental response are diametrically opposite, there is continuity in the rate of the changes induced by temperature. In other words, the relation of temperature to the *direction* or *kind* of change is discontinuous, as proved by the reversible nature of the process, but the relation of temperature to the *rate* at which these changes occur is continuous. The curves GH and IJ , drawn in relation to the base and right axes of figure 6, illustrate the writer's concept of the relation between temperature and rate of thermal induction and reversal, respectively.

The interaction of the two previously described relations (illustrated by the curves EF and GH — IJ in fig. 6) represents the writer's interpretation of the reproductive response of sugar beets to storage temperatures when such thermal exposure is followed by a favorable photoperiodic environment. It is evident that, although a large amount or high degree of thermal induction may be attained by storing sugar beets at a temperature near freezing, a long period of time is required because of the reduced rate of change that occurs at such temperatures. At higher temperatures, e. g., 6° to 8° C., the rate of induction is more rapid and the amount or degree of induction possible at such temperatures is probably adequate to induce bolting in most varieties, provided the beets are replanted in an environment affording temperatures cool enough and photoperiods long enough to prevent reversal from occurring before reproductive development is initiated. Reversal of thermal induction in plants after they are replanted in warm weather is thought to indicate another reason why Pack (12) found late planting of mother beets unfavorable for seed production.

Pack's (13) observation of complete bolting of beets after storage at 0° C. was probably due to the fact that there may have been some thermal induction in the field in the fall before harvest. Pack's dates of storage in one test are not stated, but the beets used in another

test were apparently received from Fort Collins, Colo., and experimental storage started during the latter part of November. The beets were replanted in the field at Salt Lake City, Utah, on April 1, after about 120 to 130 days of storage. Fall temperatures at Fort Collins and spring temperatures at Salt Lake City are regularly cool enough to result in considerable thermal induction before November and also after April 1. Pack, therefore, was measuring the thermal

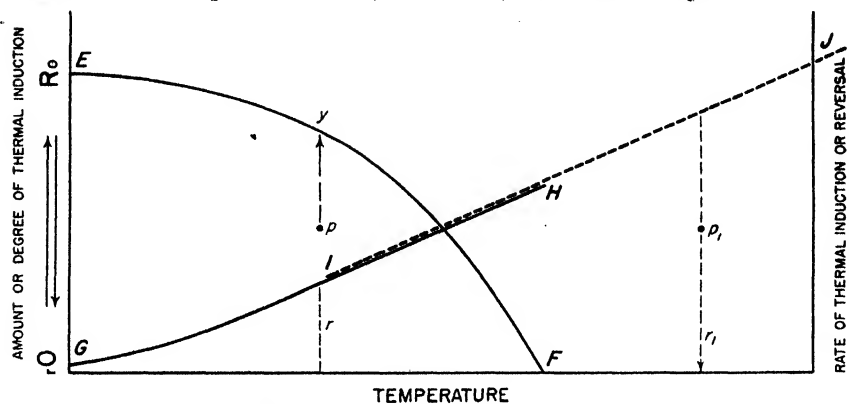


FIGURE 6.—Diagram illustrating a hypothesis concerning the separate effect of temperature on the amount or degree of thermal induction attainable and also its effect on the rate at which these thermally induced changes occur. Since the two relations have a common axis (temperature) they are illustrated in the same figure. The curve *EF*, drawn with respect to the left and base axes of the figure, indicates the relation of temperature to the amount or degree of thermal induction possible at a given temperature. Thermal induction can occur only under conditions existing within the boundary of this curve. (At temperatures higher than *F*, only substances favorable to vegetative development (*rO*) are accumulated.) The curve *GH*, drawn with respect to the base and right axes of the figure, indicates the initial rate of thermal induction at temperatures where thermal induction is possible. The curve *IJ*, which is an extension of the curve *GH*, represents the influence of temperature on the initial rate of reversal at temperatures where reversal occurs. The direction (thermal induction or reversal) of the changes that occur at temperatures illustrated by the overlapping portion of the curves *GH* and *IJ* depends upon the amount or degree of thermal induction attained before experimental storage. If beets that have previously been thermally induced to an amount or degree represented by points *p* or *p*₁ are stored at temperature *p*, thermal induction will occur at rate *r* until an amount or degree of induction represented by point *y* on the curve *EF* is approached. If a similar beet is stored at temperature *p*₁, reversal will occur at rate *r*₁ until complete reversal is effected.

induction effected both before harvest and after replanting as well as during experimental storage.

Information regarding the effects of temperature on reproduction of sugar beets as it applies to overwintering the plants in the field is of great interest in the United States, because this has become the predominant American method of sugar-beet seed growing. The fact that prevailing temperatures in some of the seed-growing areas are too warm (10) has emphasized the desirability of cooler temperatures, but Shaw's (14) results and those obtained by the writer have shown that, for optimum thermal induction, temperatures should not be too cool. From the standpoint of temperature, the most desirable seed-growing area, especially for the reproduction of non-bolting varieties, would be one in which moderately cool temperatures prevail continuously over a long period or one in which the transition

from warm to low temperatures in the fall and from low to warm temperatures in the spring is long and gradual.

The rapid rate at which reversal of thermal induction occurs at warm temperatures (tables 4 and 5 and figs. 4 and 5) suggests an explanation for the failure of nonbolting varieties to reproduce satisfactorily in some of the warmer seed-growing areas. Warm midday temperatures during even the cooler periods may so counteract the effect of favorably cool night temperatures that little net thermal induction results. Then, too, short periods of warm weather occur in these areas during late fall, winter, and early spring. Such unfavorable conditions, occurring before initiation and early development of seedstalks have been effected, may be very detrimental to seedstalk development. Tolman (16) found differences as great as 10° C. between the crown temperatures of normal and small-sized or defoliated beets during midday in February, and he showed that these temperatures were correlated with the percentage of plants that remained vegetative or produced no seed. Tolman also showed that certain cultural practices maintained a more favorable thermal environment, which resulted in an increase of plants producing seed, thereby increasing seed yields.

The fact that thermal induction is greatly retarded at temperatures near 0° C. can be utilized in breeding nonbolting varieties of sugar beets. Seed can be planted in the field in the fall; and then in late winter, after thermal induction has been completed only in the easy-bolting types, the beets can be placed in storage at -0.5° to 1.0° . Storage at such temperatures will virtually arrest further thermal induction during the remaining cool months until the beets can be replanted in the field the following spring. The easy-bolting types will then produce seedstalks and can be eliminated. This method has been used with success during two seasons by F. V. Owen⁶ and the writer. Field conditions alone do not consistently permit such effective separation of easy-bolting from nonbolting types.

The facts that thermal induction is greatly retarded at temperatures near 0° C., that the initial rate is more rapid at 6° to 9° , and that the rate of reversal of the process is greatly accelerated at about 25° suggest an explanation for the influence that certain cultural practices, designed to modify the thermal environment, also have on bolting (9, 16) and afford a better basis for devising methods to influence bolting. In beets grown for seed, complete bolting is desirable and practices favorable to it should be used; but in beets grown for sugar, bolting is objectionable and practices unfavorable to its occurrence should be followed.

SUMMARY

A measurement of the relative influence of different storage temperatures in inducing reproductive development in sugar beets was made by conducting bolting tests in a warm environment under long photoperiods. In general, beets bolted more rapidly and in larger numbers after storage at 6° to 9° C. than after storage at cooler temperatures for similar periods. Storage of beets at temperatures near 0° induced little change in the rate and percentage of plants bolting, indicating that the processes involved in thermal induction are nearly arrested

⁶ Geneticist, Division of Sugar Plant Investigations.

during such storage. Reversal of thermal induction occurred in beets that had been thermally induced previously, when those beets were stored at relatively warm temperatures (11° to 26°). The rate of both thermal induction and reversal of the process increased as the temperature was raised, the temperature coefficient of reversal being about 3.3 between 13° and 19° . The maximum amounts or degrees of thermal induction possible at the various temperatures within the favorable temperature range appear to form a gradient. Apparently there is continuity in the rate of any thermally induced change.

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ENVIRONMENTAL FACTORS AFFECTING DOWNY MILDEW OF CABBAGE¹

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INTRODUCTION

Downy mildew (*Peronospora parasitica* (Fr.) Tul.) has been reported as a serious disease of the kohl group of crucifers (*Brassica oleracea* L.) in Europe (2, 12, 15),² India (3, pp. 297-300), South Africa (6), Australia (17), and the United States (5, 8, 11, 13, 18). The disease on cabbage (*B. oleracea* var. *capitata* L.) has several phases, the severity of each depending upon current weather conditions. It commonly causes severe damage to seedlings, particularly during winter months, in the southeastern part of the United States (4, 7, 13, 21).³ Ordinarily the fungus continues to develop after transplanting but it is usually of minor importance at that time. As the crop approaches maturity under environmental conditions favorable to the mildew fungus, damage to the head may result. Bacterial soft-rot organisms may enter mildew lesions and cause damage in transit, while systemic invasion of heads in storage may lead to a dry-rot decay (16, 19).⁴

The investigation reported herein is concerned with the host range of the physiologic race of the causal organism occurring on *Brassica oleracea* in the United States and with the effect of environmental conditions, particularly temperature and host nutrition, upon development of the disease in cabbage seedlings. The studies were carried on in the greenhouse at Madison, Wis.

HOST RANGE OF THE PATHOGEN

The problem of distinguishing between the various forms of *Peronospora* which occur on members of the Cruciferae has concerned mycologists since the first description was made in 1796 by Persoon (9) as *Botrytis parasitica* Pers. on *Capsella bursa-pastoris* (L.) Medic. Fries (9) in 1849 transferred the fungus to the genus *Peronospora*, which had been established in 1837 by Corda in his description of *P. ramicis* Corda. Wilson (22) suggested a division of *P. parasitica* into three species on the basis of morphological differences in the conidia. Gäumann (9, 10) demonstrated that spore size alone was

¹ Received for publication March 15, 1944.

² Italic numbers in parentheses refer to Literature Cited, p. 80.

³ CHUPP, C. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr. Plant Dis. Bul. Sup. 26, 163 pp., illus. 1922. [Processed.]

⁴ WIAAT, J. S., and BRATLEY, C. O. DISEASES OF FRUITS AND VEGETABLES ON THE NEW YORK MARKET DURING THE MONTHS OF APRIL TO SEPTEMBER, INCLUSIVE, 1939. U. S. Dept. Agr. Plant Dis. Rpt. 23: 374-377. 1939. [Processed.]

not an adequate means of separation of species in this group, but, as a result of further morphological studies and cross-inoculation experiments on over 100 species of the Cruciferae, he published an extensive report on the physiologic races of *P. parasitica* and proposed 52 new species. In view of the uncertainty with which races of this fungus can be distinguished on any other basis than their host relations, the writers have retained the binomial *Peronospora parasitica* (Pers.) Fr.

Collections of the fungus on cabbage were secured from Florida, Louisiana, Texas, and Wisconsin. They were first inoculated to members of *Brassica oleracea*, including red and green varieties of cabbage, Brussels sprouts (*B. oleracea* var. *gemmifera* DC.), cauliflower (*B. oleracea* var. *botrytis* L.), broccoli (*B. oleracea* var. *botrytis* L.), kohlrabi (*B. oleracea* var. *gongylodes* L.), and collard (*B. oleracea* var. *viridis* L.). All of these were very susceptible to each collection of the fungus. When each collection was inoculated to radish (*Raphanus sativus* L.), turnip (*Brassica rapa* L.), rutabaga (*B. campestris* var. *napobrassica* (L.) DC.), rape (*B. napus* L.), charlock (*B. arvense* (L.) Ktze.), and black mustard (*B. nigra* (L.) Koch) lesions were produced in the form of necrotic flecks, but no spores were produced. In the case of garden cress (*Lepidium sativum* L.), stock (*Matthiola incana* var. *annua* (L.) Voss), wallflower (*Cheiranthus cheiri* L.), arabis (*Arabis alpina* L.), shepherds-purse (*Capsella bursa-pastoris* (L.) Medic.), and white mustard (*B. hirta* Moench (*B. alba* (L.) Rabenh.)), no visible signs of infection whatsoever were observed. It appeared, therefore, that the four collections belonged to a single race and that this race was confined in its successful pathogenicity to *B. oleracea* in the list of possible hosts tested. While other possible host species should be tested, it is evident that there is as yet no basis for considering wild hosts as a source of inoculum of the cabbage downy mildew organism in America. Elimination of cruciferous weeds is, therefore, not a well-founded control measure. The collection from Wisconsin was used for further experiments described in this paper.

TEMPERATURE RELATIONS OF THE FUNGUS

SPORULATION

Leaves selected late in the afternoon from inoculated plants, 2 days after symptoms had become visible but before sporulation had occurred, were chilled by placing them at 4° C. for 1 hour. They were then sprayed with distilled water from an atomizer, and six leaves, selected at random, were placed in each of seven moist chambers at 4°, 8°, 12°, 16°, 20°, 24°, and 28°, respectively. After approximately 12 hours, sporulation had occurred at temperatures of from 4° to 24°, the most abundant development of spores occurring at 12° and 16°. At 36 hours, sporulation was still greatest at 12° and 16° and nearly as profuse at 8°, but it was sparse at 4°, 20°, and 24°, and at 28° there was none. A repetition of this series with leaves on which the lesions were somewhat older gave similar results. Other observations in the greenhouse confirmed the evidence that best sporulation occurs at 8° to 16°, and it was noted further that from the lesions at these temperatures the fungus continued to sporulate longer than at other temperatures.

GERMINATION OF CONIDIA

Conidia were collected in the morning from leaves which had not previously sporulated and which were from plants that had been placed in a moist chamber the previous afternoon. This method was most satisfactory since it provided a crop of newly formed conidia. Fresh spores were desirable because it had been observed that they were extremely sensitive to aging and to desiccation. Suspensions in re-distilled water were made by removing the conidia from the sporulating lesions with a camel's-hair brush. Glass slides were treated for 24 hours in a solution of potassium dichromate in concentrated sulfuric acid, washed in distilled water, boiled for a few minutes in a solution of sodium bicarbonate, washed in several changes of distilled water, and stored in distilled water until needed. Small, moist chambers lined with water-saturated absorbent cotton were maintained in incubators at the various temperatures to be studied. Slides to be used were placed across glass rods in the bottom of each chamber. After at least 12 hours had been allowed for the moist chamber and slides to adjust to the temperature of the incubator, drops of freshly made spore suspension were placed on each slide. With these precautions little or no condensation on the slides occurred, and the drops of spore suspension remained for a week or 10 days without an appreciable change in volume. The constant-temperature incubators used were adjusted to 4° to 6°, 8° to 10°, 12°, 16°, 20°, 24°, and 28° C. A slide was taken from each chamber at various intervals, and a drop of osmic acid was added to the drops of spore suspension to kill and to fix spores and germ tubes immediately. At least 500 spores were included in each determination. The temperatures inside and immediately outside of each chamber were recorded with each determination.

Several experiments were run with similar results. The data from a typical one are presented in figure 1. A high percentage of germination occurred promptly at all temperatures between 6° and 16° C. Germination was very much reduced at 20°, very meager at 24°, and none occurred at 28°. It may be seen, however, that the initiation of germination was decidedly the most rapid between 8° and 12°, where nearly 60 percent had germinated within 3 hours, while less than 20 percent germination was recorded at all other temperatures at this interval. This is, of course, a very important factor when the period of high humidity conducive to germination is short. At intervals above 8 hours there was little difference in the percentage of total germination at temperatures of 16° or below. In fact, there was a tendency for the highest percentage of germination to occur at the lowest temperature, which in this case was about 6°.

PENETRATION

Leaves of comparable ages were picked from young cabbage plants, and six, selected at random, were placed in each of six moist chambers, which were placed in incubators adjusted to 4°, 8°, 12°, 16°, 20°, and 24° C., respectively. After 24 hours several drops of spore suspension were placed on the lower side of each leaf. Leaves were removed frequently from each chamber and examined for the percentage of germination, formation of appressoria, penetration, col-

lapse of conidia, and formation of haustoria. The results are given in figure 2. Germ tubes appeared first at 12° and 16° and next at 8°. Appressoria formed first at 16° and next at 12°, 20°, and 24°. Penetration occurred first at 16°, next at 20° and 24°, and spores collapsed first at these three temperatures. Haustoria formed first 5 hours

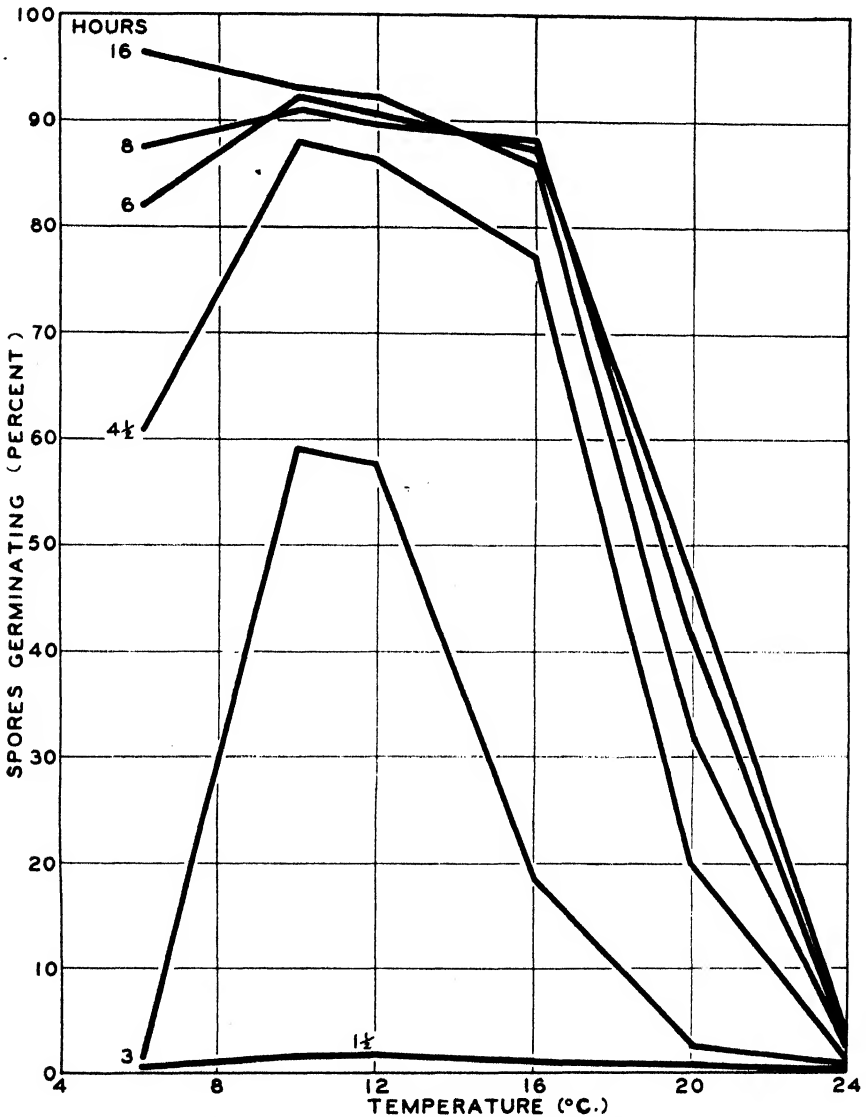


FIGURE 1.—Effect of time and temperature on germination of conidia.

after inoculation at 16° and 20°, and an hour later at 24°. Thus, while temperatures between 8° and 12° showed the most rapid germination as measured by percentage of spores germinating, the process of appressorial formation and penetration was optimum at

16°. Moreover, penetration and development of haustoria proceeded more promptly at 20° and 24° than at 12°, and the progress was decidedly delayed at 8° and 4°. After 22 hours the haustoria were

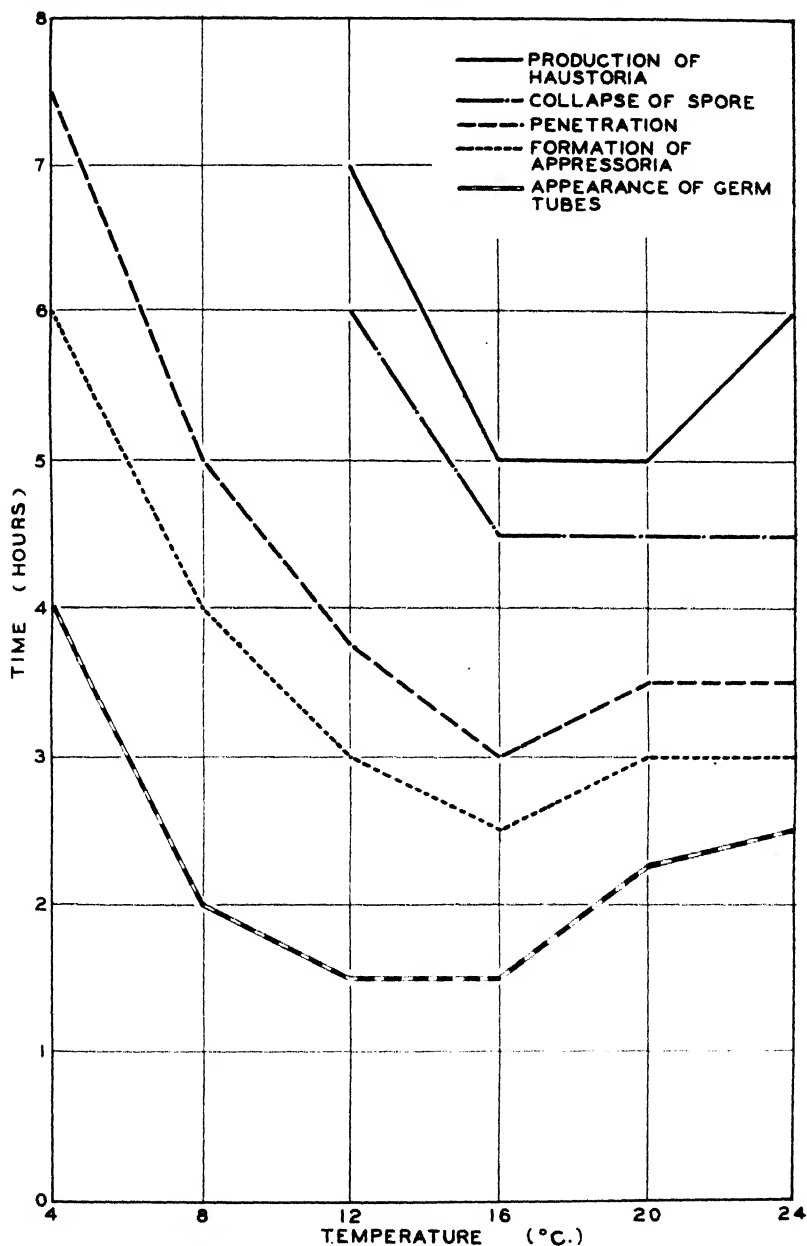


FIGURE 2.—Effect of temperature upon penetration of fungus and development of haustoria.

most abundant, and the diameter of the mycelium was greatest at 16°. The development of the fungus was next most vigorous at 20°.

RELATION OF TEMPERATURE AND HUMIDITY TO DISEASE DEVELOPMENT

CONSTANT TEMPERATURES AND HUMIDITIES

The development of mildew was studied first at constant temperatures and high humidity on young seedlings. Four flats of plants just showing a third leaf were inoculated with a suspension of spores. After 20 hours at 15° C. in a moist chamber, chlorotic spots with faintly flecked centers marked the points of infection on the first and second leaves. At this stage one flat each was placed in greenhouses at 16°, 20°, 24°, and 28°, respectively. Three days after inoculation cotyledons at 24° had begun to wilt, and leaf lesions were distinctly more advanced than at 20° or 28°; little progress was evident at 16°. At 4 days the cotyledons at 24° had become desiccated, and lesions were more pronounced on the hypocotyl and leaves. The disease continued to progress most rapidly at this temperature, followed in descending order of severity by temperatures of 28°, 20°, and 16°.

The experiment was repeated with potted plants having six to eight leaves. After inoculation and a 24-hour incubation in a moist chamber at 15° C., some plants were placed on an open bench in relatively dry atmosphere, and others were kept under a cloth moist chamber in which the air was kept saturated by means of a fine spray for an atomizer. The temperatures at high humidity were 12°, 16°, 20°, and 24° C., respectively, while those at low humidity were 16°, 20°, 24°, and 28°. Symptoms were evident at low humidity at 20°, 24°, and 28° on the fourth day after inoculation and on the fifth day at 16°. Again the disease progressed most rapidly at 24° and nearly as rapidly at 28° while the affected leaves dropped promptly at these high temperatures. At high humidity the disease developed most rapidly, and sporulation was abundant at 24°, while at this temperature and at 20° the tissue on which sporulation occurred was invaded promptly by secondary organisms.

In a third experiment plants at the fifth-leaf stage were used. The same temperatures at low humidity were provided; at high humidity the temperatures were 12°, 16°, 19°, 23°, and 28° C. The results are presented graphically in figure 3. Since the presence of spores actually marks the completion of the cycle in the host, their appearance is the most suitable character for use in determining the incubation period. Furthermore, the development of symptoms may be influenced by the effect of relative humidity, sunlight, soil moisture, and other factors on the host, while sporulation is a direct indication of the progress of the fungus. The disease appeared first at 24° at low humidity and next at 23° at high humidity. Initial sporulation appeared first at 23° at high humidity. The lesions at 20° and 24° were darkest in color; those at 28° showed a pronounced yellow halo.

The three experiments demonstrated that progress of the fungus in the host tissue was most rapid at about 23° C. At high humidity the cycle from conidium to conidium was shortest at this temperature, although, as shown earlier, sporulation was most abundant at from 8° to 16°. The appearance of symptoms was also most prompt at 23° or 24°. The fact that the first signs appeared some 12 hours earlier at low humidity is due to the fact that invaded tissues collapse more rapidly at low than at high humidity and the lesions thus become visible more promptly.

ALTERNATE LOW AND HIGH HUMIDITY

Natural climate often consists in alternating periods of high and low humidity. To approximate such a condition with controlled temperature, cabbage plants selected for uniformity of size and vigor were inoculated in the moist chamber at 16° C., and 5 days later when

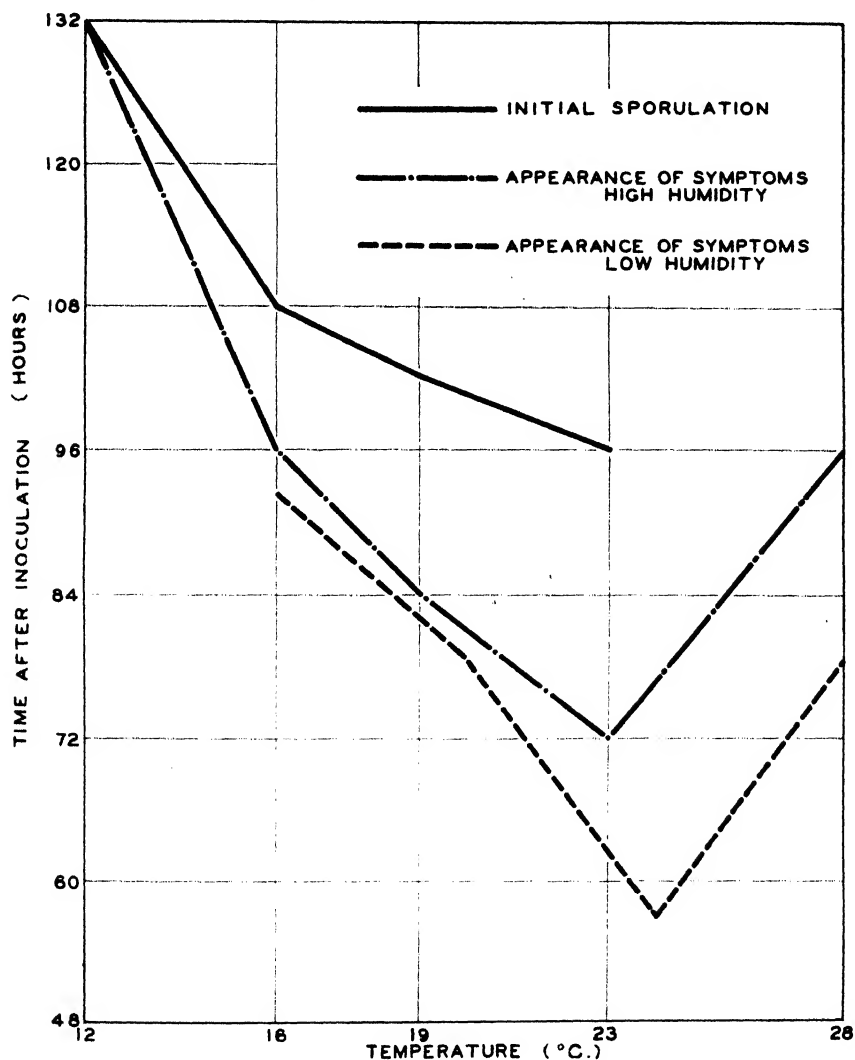


FIGURE 3.—Effect of five different temperatures on the initial sporulation of fungus at high humidity and upon initial appearance of symptoms at low and at high humidity.

heavy and uniform infection became evident, they were transferred to greenhouses at 16°, 20°, 24°, and 28°. Twelve plants were placed in a moist chamber and six on an open bench in each house. The plants in the moist chambers were subjected to alternate wet and dry periods of 24 hours each. During the periods when the chambers

were sprayed with water, their temperatures ran 4 to 5 degrees lower than that of the respective houses in which they were kept, while during the dry periods the temperatures in the open moist chamber and in the remainder of the house were approximately equal. This treatment continued over a period of 4 weeks. To simulate natural conditions and bring about spore dispersal, the atomizer spray in each moist chamber was directed at the plants.

At 28° C., sporulation was abundant in the moist chamber during the first two treatments but rapidly decreased in subsequent moist periods as the infected leaves dried and fell. After 4 weeks a few small static lesions from the original inoculation remained, but no new lesions were evident. The plants on the open bench had lost all infected leaves. At 24° sporulation also decreased rapidly as early defoliation took place. A small amount of secondary infection was evident after 10 days, but at the end of 4 weeks there were no additional lesions and the spots resulting from secondary infection did not produce spores. Some remaining lower leaves showing original infection produced a few conidiophores at the margins of old lesions. The plants on the bench also retained leaves showing static lesions. At 20°, sporulation rapidly declined after the first week. There was, however, a considerable amount of secondary infection producing lesions with normal sporulation. All plants retained leaves infected by the original inoculation, and in some of these lesions the fungus sporulated to a slight degree along their margins. The plants on the bench retained fewer of the originally infected leaves. At 16°, sporulation was abundant up to the time the plants were discarded, spores being produced on new as well as old lesions. Thus, the disease was most active by far at 16° because of the presence of new lesions and the continued activity of old lesions. The prolonged sporulation which followed the alternate dry and moist periods might have been due in part to the low temperatures during the moist treatments.

Upon the repetition of this experiment, but with a moist period of 48 hours only once a week, the results were essentially the same. Secondary infection was again sparse at the three highest temperatures although sporulation was uniformly heavy at first. The inoculum was soon depleted by the rapid drying of the infected leaves before additional leaves had reached stages suitable for infection. Lesions which did occur at 24° and 28° C. on young leaves were small and inactive. At 16° new infection had taken place, and old lesions were still active.

The effect of temperature at alternate periods of low and high humidity was similar to that at constant humidities. As the temperature was raised, the rate of mycelial growth and development increased up to 24° C. but was retarded somewhat at 28°. However, temperatures of 20° and above were less favorable to disease development since the active sporulating period of leaf lesions was greatly shortened and germination of spores was reduced. It was apparent that at 20°, 24°, and 28° the host rapidly outgrew the effects of infection, for as the infected leaves quickly desiccated they became ineffective as sources of inoculum. While the fungus developed more slowly at lower temperatures (12° and 16°), the period of effective sporulation was greatly lengthened, and the reduced growth rate of the host permitted heavier and more effective infection.

The alternation of periods of high and low humidity had little effect on the rate of desiccation of lesions at 24° and 28° C. However, at 16° the treatment successfully maintained the disease. It seems probable that the early recovery of plants from the effects of the disease when temperatures were above 20° was in part the result of the greatly shortened period of activity of the fungus in the lesions and the increased growth rate of the host.

RELATION OF HOST NUTRITION TO DISEASE DEVELOPMENT

The reports of increased or reduced susceptibility of cabbage and other crucifers to downy mildew resulting from variation in host nutrition are all based on field observation. Quanjer (15) stated that cauliflower plants showing marked potash deficiency symptoms were severely attacked by *Peronospora parasitica*, while those fertilized with potash were but slightly attacked. Chupp (4) found considerable reduction of tipburn but a marked increase in mildew where potassium had been added. Fertilization with nitrates also increased the severity of mildew, but phosphates seemed to decrease the amount of disease. Chupp pointed out that the experimental data were too limited to warrant definite conclusions but that the differences in disease development were striking. Townsend (20) obtained results which indicated that cabbage plants grown in soil treated with phosphorous were more subject while those in soil treated with potash were less subject to the disease than plants grown on unfertilized soil. Brejneff (1) reported very little difference in downy mildew incidence on crucifers grown with various applications of fertilizers. De Bruyn (2) found that nutrition had little effect on susceptibility except where it influenced the vigor and condition of the leaves. Chlorotic leaves approaching senescence were much more susceptible than vigorous ones, although plants were more susceptible during the summer when light was stronger and of longer duration. She concluded that the influence of soil fertility was secondary to that of the vigor of the foliage.

The conflicting evidence just cited indicated that a profitable study might be made of the effect of nutrition on downy mildew. A constant drip method, already described by Pryor (14), for applying nutrient solutions to pure quartz sand was adopted for the present investigations. Each series of plants was given 8 different nutrient treatments: Excesses and deficiencies of nitrogen, potash, and phosphorous; deficiency of sulfur; and a balanced nutrient. Cabbage seedlings were grown to the first-leaf stage in sterile quartz sand and 20 were transplanted to each pot. Three or 4 pots were used in each treatment. When the plants were 7 to 9 inches tall and showed from 8 to 10 leaves, they were inoculated simultaneously in a large chamber and returned after 24 hours to the drip system. Disease readings were taken after symptoms had fully developed. The number of lesions on mature leaves and their relative size were estimated and rated according to severity in classes ranging from 1 to 10, and a disease index was obtained by multiplying lesion-spread by lesion-number ratings. A sporulation index was used to indicate the proportion of the lesions which sporulated after the period allowed for development. A plant-size index was also employed with 1 representing the smallest size of plant in any given series and 10 the largest.

The plants in the first experiment were placed in the moist chamber on the morning of March 2 and were inoculated at 12° to 15° C. with a uniform suspension of freshly produced spores late the same evening. The moist treatment was continued until the following afternoon, and additional 24-hour moist treatments were provided on the third, fifth, and ninth days. The readings were made on the tenth day following inoculation when the more severely diseased leaves were almost entirely permeated by the fungus. The second experiment, started on May 15, was conducted similarly except that the inoculation temperatures were between 15° and 17°. This series was reinocu-

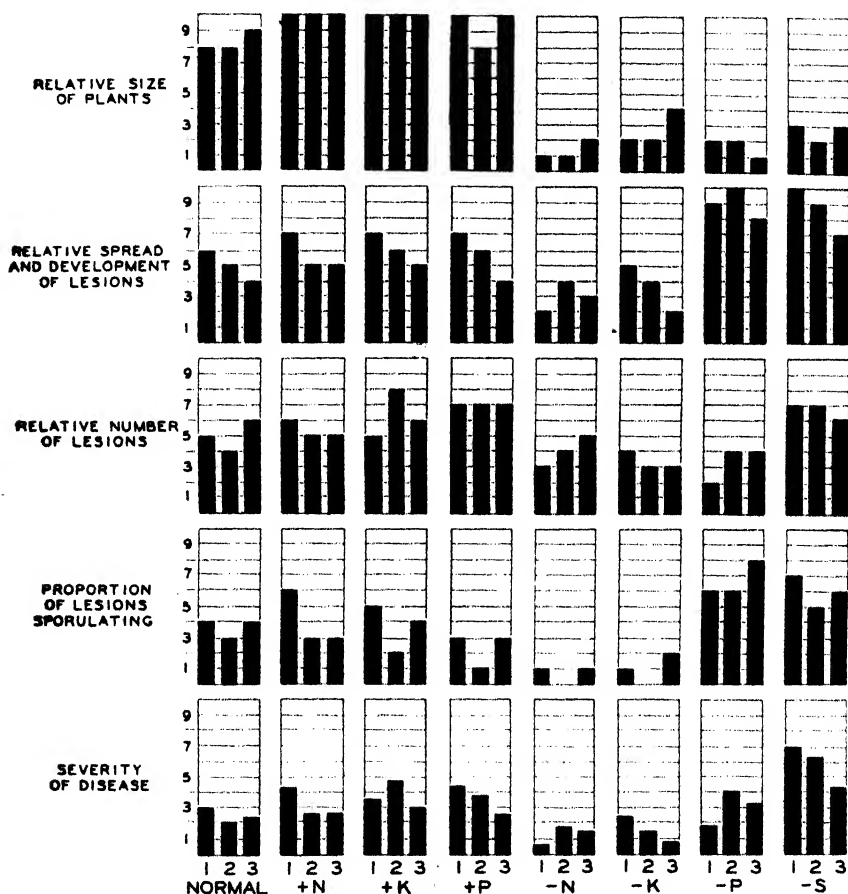


FIGURE 4.—Graphic summary of infection by and development of *Peronospora parasitica* on cabbage plants grown in sand culture supplied with various nutrient solutions.

lated on the second day, and a moist treatment was provided on the ninth day. Results were recorded 10 days after inoculation. In general, the number of lesions was slightly lower in this experiment than in others, probably because of the low vitality of the spores in the suspension used for inoculation. The third experiment was run during July when temperature was difficult to control. The moist

chamber varied between 15° and 18° during the 20-hour inoculation period. The day temperature of the greenhouse was about 32° and early drying of the diseased leaves occurred. Infection was heavy and records were taken 8 days after inoculation.

Examination of the data from these three experiments reveals differences between the various nutrient treatments both in number and type of lesions. The results are presented graphically in figure 4. While the plants in the deficiency solutions were very stunted, certain differences appeared consistently. The relative number of lesions developing on the plants deficient in N, K, and P was considerably less than on plants deficient in S and on those having an excess of N, K, and P. A difference in amount of bloom on the leaves might explain part of the variation. In the -P and -S series the lateral spread of the fungus and the amount of sporulation were markedly greater than in the plants of comparable size of the -K and -N series, and noticeably more than in the larger plants of the series with normal nutrition or with excessive amounts of N, P, or K. In the -P series the greater development of the fungus as compared with that in other series was associated with greater thickness of the crinkled, stunted leaves.

Since there was reduction in size of plants with all deficient solutions there was no correlation between susceptibility and vigor as indicated by size or rate of growth. In general, lesions developed slowly on immature leaves and very rapidly on senescent leaves. Physiologically old leaves tended to develop more rapidly in -P and -S solutions than in -N and -K solutions and this seemed to account for the greater spread of lesions and more abundant sporulation on plants in the first two solutions.

The previously noted relation between deficient chlorophyll and susceptibility was not apparent in these experiments. Leaves in the -S and -N series were quite yellow, those in the -K series were bluish green, and those in the -P series were light green. The plants grown with excessive concentrations of N, P, or K showed little or no difference in either number or spread of lesions.

A fourth experiment was conducted in which the concentration of the basal solution was increased 10 times and 30 times. On June 1, when the plants had from six to eight leaves, they were inoculated. Little difference in vigor of plants at the three concentrations was evident until the time of inoculation, when plants in the highest concentration had attained growth beyond the optimum. Infection was uniformly good, but the disease developed most severely on the plants at the highest salt concentration. There was little difference in disease development between the lowest and the intermediate concentrations.

DISCUSSION AND SUMMARY

The severity of downy mildew on young cabbage seedlings is greatest in areas in the United States where the plants grow in seedbeds throughout protracted cool periods during which relative humidity is high. According to Eddins (7), the disease is most destructive in Florida when the temperature ranges between 10° and 15° C., and when the plants remain wet until midmorning for 4 days. The studies reported herein show that the fungus sporulates most readily at a

range of 8° to 16°, the conidia germinate most rapidly at 8° to 12°, and penetration of the host occurs most rapidly at 16°. Thus, these stages of the life cycle of the fungus which are essential to the development of inoculum and to infection are favored by relatively low temperatures.

After invasion of the host, haustoria grow most rapidly at 20° and 24° C. When disease development was studied at a range of temperatures, it was found that the symptoms developed most rapidly at 24°. When humidity was high, the lesions which developed first at this temperature also sporulated first. However, the disease ran its course most rapidly at the temperature most favorable for mycelial spread within the host. Sporulation and reinfection were limited at 24° and at 28°, and an increased growth rate of the host resulted in more rapid maturation and dropping of the lower leaves. At the low temperature (16°) the growth of host and fungus was slower, but sporulation and reinfection were much more pronounced. Here the fungus was most prolific, and the disease developed most profusely.

It is thus apparent that the low optima for sporulation, germination, and penetration are more important to disease development than the higher optimum for growth of the fungus. The severity of the disease at about 10° to 15° seems to be best explained by the effect of temperature upon production of inoculum, spore germination, and infection.

The confusing observations recorded in the literature on the effect of fertilizers on the development of mildew were not cleared up by the study of disease development on plants grown in a range of controlled nutrients. In fact, the results would indicate that little benefit could be expected in the control of mildew through adjustment of fertilization of seedbeds.

The commonly cited recommendation of eradication of cruciferous weeds to control this disease is of no value in view of the fact that the collections made of the fungus from widely separated localities in the United States were apparently all of one physiologic race. This race was confined in its pathogenicity to members of *Brassica oleracea*, of which none grow in the wild state in this country.

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DIFFERENTIAL BACTERICIDAL ACTIVITY OF BOVINE SERUM TOWARD STRAINS OF BRUCELLA ABORTUS OF HIGH AND LOW VIRULENCE¹

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INTRODUCTION

The discovery by Bordet (3)³ that the bactericidal action of serum depends upon two elements, now known as immune body and complement, was followed by intensive study of these phenomena. Much of the earlier literature has been reviewed by Muir and Browning (14), and an extensive reference to later work has been given by Dingle, Fothergill, and Chandler (5). The role of complement in immunological phenomena has been reviewed in the excellent treatise by Osborn (15).

Perhaps the majority of the studies have been made with the primary purpose of explaining the mechanism of immune processes. There have been some, however, which attempted to correlate the bactericidal or virucidal activity of whole blood or serum with variation in the virulence of a pathogen, or to determine whether such activity was associated with resistance or susceptibility of a host to a particular infectious agent. For example, a report by Malone, Avari, and Naidu (13) has shown that in the black rat (*Rattus rattus*) there was a significant and positive correlation between the bactericidal power of whole blood and resistance to *Bacillus pestis*. In an attempt to determine the nature of inherited resistance to *Salmonella enteritidis* in the laboratory rat (*R. norvegicus*), Irwin and Hughes (11) obtained a similar positive correlation between antibacterial action and resistance. Furthermore, preliminary results by these authors (10) suggested that the difference between individuals (in rats) in antibody content was determined in part by heritable factors. Bull and Tao (4) report their own and cite other studies as indicating an association of bactericidal activity of whole blood and resistance in rabbits, and in chickens as compared with rabbits, to pneumococcal infections. The experimental results of Silverthorne (17) and Silverthorne and Fraser (18) indicate that the bactericidal power of whole blood of human beings and guinea pigs against various strains of meningococci is correlated with the virulence of these organisms and with protection after immunization.

Studies on the relation of the age incidence in human beings to the antibacterial activity of whole blood against pneumococci were reported by Sutliff and Finland (19) and against *Haemophilus in-*

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³ Italic numbers in parentheses refer to Literature Cited, p. 90.

fluenzae by Fothergill and Wright (7). The findings of these authors were in agreement in showing that the bactericidal power of the blood of children at an early age was relatively low or absent, and that there was an increase of this activity with advance in age. The technique has been widely employed in studies of pneumococcal infections. Ward (20) found that the whole blood of individuals (human) varied considerably in activity toward different types of pneumococci. Dozois, Seifter, and Ecker (6) have stated:

Although much of the work on bactericidal and hemolytic activities is contradictory, it has been firmly established that these activities depend on the interaction of a thermostable antibody and a thermolabile complement. Only the thermostable antibody is increased by immunization. Neither substance by itself has bactericidal or lytic effects.

It has previously been shown by Irwin and Ferguson (9) that bactericidal antibodies ("bactericidins") of high titer were present in the serums of cattle which had recovered from an induced infection of *Brucella abortus*, and whose serums had ceased to react in agglutination with the organism beyond the range of serums from normal cows. The studies reported in this paper were made to test whether (1) the serums of cows vaccinated with strain 19 of *Br. abortus* would exhibit parallel phenomena, and (2) whether there would be a differential activity of such serums toward strain 19 and one of greater virulence.

MATERIALS AND METHODS

On September 23, 1940, 15 cows varying in age from a minimum of 18 months were vaccinated subcutaneously with 5 cc. of *Brucella abortus*, strain 19. These animals were offspring of the cows that had made up herd 3, which has been previously described (1, 2). They had been bred 1 to 3 months prior to vaccination.

Previous experience⁴ in this laboratory in measuring the bactericidal effect of serums which had a titer of agglutinating antibodies beyond 1:100 for *Br. abortus* had shown that a definitely higher titer of antibodies with antibacterial action ("bactericidins") than of agglutinating antibodies could usually be demonstrated in such serums. However, since the serum of infected animals from whose blood the agglutinins had practically or entirely disappeared still contained demonstrable bactericidins for *Br. abortus*, as previously reported (9), comprehensive tests on the bactericidal activity of the serums of these vaccinated animals were delayed until the agglutinating titer of their respective serums was usually 1:100 or less. The tests to be reported in this paper were made on the serums of the animals between 6 and 18 months after the vaccination.

The organisms used in these tests were cultures of *Br. abortus* of strain 19 and a virulent strain, No. 1242⁵. Shortly after the experiments were started, tests⁶ for the virulence of strain No. 1242 were made on two isolates from single colonies, by the usual method of injection into guinea pigs. Each of six animals in the group injected with organisms of one or the other of the isolates showed a high titer of agglutination and definite lesions, but of varying degrees, in the

⁴ By the authors and Dr. L. C. Ferguson.

⁵ Both strains were obtained through the courtesy of Drs. A. B. Crawford and Adolph Eichhorn of the Animal Disease Station, Bureau of Animal Industry, U. S. Department of Agriculture.

⁶ The writers are indebted to Mrs. Mildred M. Johnson for making the tests of the virulence of these isolates.

spleen. These results were taken as a reasonable index of the virulence of the isolates. Care was exercised in culturing both strains to avoid the propagation of variants.

Previous trials of the bactericidal activity of whole blood, plasma, serum, and white blood cells alone have shown that the primary, if not the entire, bactericidal properties of cattle blood toward *Br. abortus* are found in the serum (8). (White blood cells alone or in combination with the red cells had no bactericidal effect whatever on *Br. abortus*. As previously stated (8), serum alone, even from cows whose whole blood had very little antibacterial activity, showed more bactericidal action toward *Br. abortus* than did whole blood.)

Essentially the same technique was employed in making the tests of the bactericidal activity of serum from the various animals as has been previously described (8, 9, 16). Blood was taken under aseptic conditions from the jugular vein of the animals into tubes, and allowed to clot. The tubes usually stood at room temperature for about an hour, and were then stored in a refrigerator until the following morning. Beef serum diluted more than 1:5 usually shows very little if any antibacterial action for *Br. abortus*, unless complement from beef serum is added. As has been reported by Shrigley and Irwin (16), complement from guinea pigs or rabbits is incapable of activating beef serum against *Br. abortus*, so the complement in these tests was obtained from cattle not infected with *Br. abortus* (i. e., "normal" animals). Considerable differences in activity of the complement from different individuals have been encountered, and, insofar as was possible, serum from one or the other of two individuals was used as the source of complement in the tests. The complement was prepared by absorbing serum from defibrinated blood with heat-killed cells of *Br. abortus* (0.3 cc. of bacteria per 10 cc. of serum), at 0° C. for 20 to 30 minutes. The antibodies, but not the complement, of the serum are absorbed at this temperature. The absorption was usually done in late afternoon, and the complement was stored overnight in a refrigerator after being removed from the bacterial serum mixture. Centrifugation of the mixture was more quickly done with an "angle centrifuge" than with the usual type.

In the bactericidal set-up there was a series of tubes (pyrex) for each serum and the different dilutions of each serum to be tested. These tubes contained, individually, 0.05 cc. of a decimal dilution of a standardized suspension of a 36- or 48-hour culture of *Br. abortus*, either strain 19 or 1242. The organisms were washed off the slant in saline, and the volume was adjusted to correspond approximately in number with a standard suspension. From this, the first dilution was made, 10^{-1} . The successive dilutions are termed 10^{-1} , 10^{-2} , . . . 10^{-7} , respectively, according to general usage. The dilutions were made in broth so as to avoid any antagonistic action of saline on the organisms. To each of the tubes in the series was then added 0.3 cc. of serum, or of the dilutions of serum; to the tubes containing diluted serum was also added 0.2 cc. of the complement. Following incubation at 37° C. for 22 to 24 hours, the contents of each tube were poured into a pyrex Petri dish with fluid tryptose agar and incubated 5 or 6 days. The number of colonies in each plate was then compared with that in the bacterial controls. As a control in each set-up on the activity of the complement, 0.3 cc. of serum which had been heated at 56° C. for 30

minutes, complement (0.3 cc.) alone, and the combination of heated serum (0.3 cc.) and complement (0.2 cc.) were tested in the same manner as outlined above for the serum. Only those tests in which the complement restored bactericidal activity to heated serum approximately equivalent to that of the undiluted serum were considered to be satisfactory and have been included in the data to be presented.

For the bacterial control, 0.05 cc. of each of the bacterial dilutions was plated out directly in fluid tryptose agar, and the number of colonies in the two highest dilutions was counted after incubation. Usually duplicate samples were made of the two highest dilutions. Also, 0.05 cc. of each of the three or four highest dilutions of bacteria was placed in broth, incubated, and then plated with the others. The colonies from the highest dilution of bacteria (10^{-7}), when grown in broth, were always too numerous to be counted, as compared with an average number of 10 in the control.

Thus in the test as a whole, the ability of the organisms to grow was determined by seeding from each of the higher dilutions into broth, and the approximate number from each dilution placed in the respective tubes to be tested with the serum and its dilutions was measured by the bacterial control. The number of organisms in the series of plates testing the bactericidal activity of heated serum was usually slightly less than that of the bacterial control, indicating probably a minimum inhibition of growth by the heated serum. If there was little or no bactericidal activity in the tests on the complement alone, it was concluded that the absorption of antibodies was adequate and the addition of complement to the various dilutions of serum did not by itself produce any antibacterial activity. All these factors were satisfactorily controlled before the test at any time on any animal was considered to be adequate.

EXPERIMENTAL RESULTS

The antibacterial action of the serum and dilutions of serum was estimated by contrasting the number of colonies in the tryptose agar controls with those in the organism-serum mixtures. An index of the bactericidal activity of the serum, or dilution of serum, of any individual was calculated in the manner suggested by Mackie and Finkelstein (12), by subtracting the last (bacterial) dilution in which growth occurred in the serum mixtures from the last dilution in which growth occurred in the control. Thus, as is shown in table 1, the undiluted serum of cow No. 68 as a rule allowed slight growth of strain 19 in the -2 dilution, none in the -3. However, the bacterial control showed a mean number of colonies of between 10 and 20 in the -7 dilution, and the bactericidal index of these tests is then 5. The average number of colonies, in all the tests made, in the highest dilution (-7) of bacteria was between 10 and 20. That in the next lower dilution (-6) was between 100 and 200.

Thus, 0.3 cc. of serum from this normal animal was able to destroy somewhat more than 100,000 bacteria of strain 19, a bactericidal index of 5. As is shown in table 1, in the same number of tests, made at the same time, the bactericidal index toward the virulent strain (No. 1242) of *Br. abortus* was 4, or more than 10,000, but less than 100,000 organisms killed by the same amount of serum which destroyed more than 10^5 organisms of strain 19. The bactericidal

indices for the various dilutions of serum of cow No. 68 against strains 19 and 1242, respectively, as shown in the table, are as follows: For serum diluted 1:10, the index was 5 for strain 19, 2 for 1242; at 1:20, an index of 3 for each strain; at 1:40, 3 for 19 and 1 for 1242; at 1:80, 2 for strain 19 and 1 for 1242; at 1:160, 2 for strain 19 and none for 1242; and at 1:320, no definite antibacterial activity for either strain.

TABLE 1.—Summary of several tests on the antibacterial action of the serum of a normal animal (cow No. 68) against 2 strains of *Brucella abortus*

Serum	Strain of <i>Brucella</i> tested	Number of tests	Bacterial dilutions ¹						Bactericidal index
			10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	
Undiluted serum	19	9	+	0	0	0	0	0	5
	1242	9	++	++	0	0	0	0	4
Serum diluted:									
1:10	19	6	++	0	0	0	0	0	5
	1242	6	+++	+++	++	+	0	0	2
1:20	19	3	+++	+++	++	3	0	0	3
	1242	3	+++	+++	+	0	0	0	3
1:40	19	6	+++	+++	+	0	0	0	3
	1242	6	+++	+++	++	+	+	0	1
1:80	19	3	+++	+++	++	+	0	0	2
	1242	3	+++	+++	++	++	+	0	1
1:160	19	6	+++	+++	+++	++	0	0	2
	1242	6	+++	+++	+++	+++	+	+	0
1:320	19	3	+++	+++	+++	+++	+	+	0
	1242	3	+++	+++	+++	+++	+++	+	0
Heated serum + complement	19	8	++	+	0	0	0	0	4
	1242	9	+++	+++	++	0	0	0	3
Average number of organisms in the dilutions of the bacterial controls			10 ⁶	10 ⁵	10 ⁴	10 ³	10 ²	10	—

¹ 0 indicates that there were no colonies in the combination of serum and bacterial dilution; + indicates the presence of a few colonies in some or all of the tests; ++, somewhat more colonies than indicated by +, but less than the bacterial control; +++, an appreciable number of colonies, but not necessarily as many as in the bacterial control.

Except for differences between individuals, the results observed with the serum of this animal are typical of those obtained with several other normal animals which have been tested in this manner. Usually little if any antibacterial activity toward either bacterial strain was shown by the serum of any normal animal at a dilution of 1:160. Occasionally an animal has been tested whose serum showed little or no bactericidal activity beyond a dilution of 1:10.

The reactivity of the serum of cow 68 was somewhat greater toward strain 19 than toward 1242 in the majority of these tests, as is indicated by the averages given in table 1. Only infrequently did the converse situation obtain. This differential action toward the two strains, themselves differing in virulence, was usually, but not always, encountered in repeated tests of a few other normal animals. In the majority of such tests, however, wherever there was a difference in reactivity of the serum toward the two strains of the organism, it was definitely in the direction of a greater number of organisms of strain 19 being acted upon than of the other.

According to these results, the serum of a normal animal rarely, if ever, exhibits bactericidal activity toward strain 19 of *Br. abortus*, or toward a virulent strain, beyond a dilution of 1:160. Repeated tests of such activity toward virulent strains were made at an earlier date on the serum of animals of herd 3, prior to their artificial exposure as previously described (1, 2). The serums of only a few of the normal

animals of this group showed an antibacterial action at dilutions of 1:160, those of the majority had a maximum bactericidal titer of 1:40 or 1:80. Therefore, any increase in the titer of the bactericidins, following infection or vaccination, over the maximum observed for normal animals, may almost certainly be attributed to the stimulation induced by the infection or vaccination.

Data on tests performed with the serum of 12 vaccinated animals are presented in table 2. The digits given in the table represent the average bactericidal indices for the serum and various serum dilutions of the animals listed, toward each bacterial strain. The average indices are listed, rather than the results of single trials, as these should give for any individual a more accurate measurement of differential action toward the two strains of *Brucella* than any single trial. On the other hand, fluctuations in the titer of the bactericidins, particularly the anticipated decrease with time, would tend to be concealed in an average index, depending upon the time elapsing between tests on an individual.

TABLE 2.—The average bactericidal indices, toward 2 strains of *Brucella abortus*, of the serums of 12 cows vaccinated at the age of 18 months or more with a culture of strain 19

Cow No.	Strain of <i>Brucella</i> tested	Average indices ¹ of bactericidal activity of--										Agglutination titer
		Undiluted serum	Serum dilutions at--								Heated serum + complement	
			1:10	1:40	1:160	1:640	1:1,280	1:2,560	1:5,120	1:10,240		
15E	19	4.2	4.7	3.3	2.7	3	3.5	4	2.5		3.7	Partial at
	1242	3	2.5	1.5	1.5	2.3	3	2.3	1.5		2.5	1:100.
20E	19	6	5	4.3	4	4	4	2.7	1.7		4.7	Partial at
	1242	3.3	3	2.3	2.3	2.3	2.3	2	1		2.3	1:100.
1F	19	5.3	4.7	3	5	3.7	4	3	3		6	Partial at
	1242	1.7	2	1.7	3	2.7	3	3	2		4.3	1:50.
63F	19	6	5	5	4	4		3	1	0	5	Partial at
	1242	4	2	2	2	1		1	0	0	3	1:100.
2g	19	6	4.5	4.5	4	3		2	2	2	6	Partial at
	1242	5	3	3	3.5	2.5		0	0	0	4	1:50.
6g	19	4.7	3.3	2.7	3	2.7		1.7	1	1	3.3	Slight at
	1242	4	2.7	2.7	2.3	2		1.7	1	0	2.7	1:200.
8g	19	6	5.3	4.7	4	2	2.5	1	.7	0	4.3	Slight at
	1242	4	3	2.3	2.7	2.5	2	.5	.3	0	3	1:100.
11g	19	5	4.3	4.7	4	4.3	3.5	3.3	3	4	5	Slight at
	1242	3	3.7	3.3	3	2.7	2	2.3	2	1	4	1:25.
25g	19	6	5.7	5	4	4	3	2	1		3.3	Partial at
	1242	3.7	2.7	1.7	2.3	2.7	2.7	1.7	.7		3	1:100.
33g	19	6	5	4.5	4	3.5	3	2.5	2		5.5	Slight at
	1242	4.5	4	3	2.5	2	2	1	0		3	1:50.
38g	19	5.7	5	4.2	3.5	3.5	4	3.5	1.7		4	Slight at
	1242	3.2	2	2	2	2	2	1.7	1		2.5	1:100.
63g	19	5.7	5.3	4.3	4	3	2.3	.5	.3		4.7	Slight at
	1242	3.7	3.3	3	2.5	3	1.3	.5	0		3.7	1:50.

¹ The digits represent the average index of bactericidal activity at the various dilutions of serum. (See text for explanation.) The average number of organisms of either strain at the highest dilution (10^{-7}) was between 10 and 20, and at the next highest dilution, between 100 and 200. Thus 0 indicates no organisms destroyed; 1, more than 10 but less than 100; 2, more than 100 but less than 1,000, etc., the indices corresponding approximately to the respective powers of 10 in number of organisms acted upon.

In these tests, the average number of organisms in the 10^{-7} dilution was 10 to 20, so an index of 1 for a particular serum dilution would represent no colonies present in that bacterial dilution. If colonies were present in this bacterial dilution of controls for heated serum and complement, the conclusion would be strengthened that the action of the serum dilutions and complement is definitely bactericidal. In the writers' opinion, however, an index of 1 is hardly large enough

to be considered as significant; an index of 2, indicating that 100 or more organisms were destroyed, may be taken as indicative of a slight, but appreciable, bactericidal action. The indices correspond to the respective powers of 10 in the minimum number of organisms killed. Thus, in addition to the number of organisms destroyed if the indices were 1 and 2, an index of 3 represents 10^3 or 1,000 as the minimum number killed, an index of 4= 10^4 or 10,000, etc.

In table 2, it may be seen that cow No. 15E showed a definite index of bactericidal activity in the undiluted serum and in the various dilutions through 1:5,120. These indices are averages of four tests, one made in June 1941, one in January and two in March 1942. Only in June 1941 and in January 1942 were the serum dilutions of 1:2,560 and 1:5,120 included in the tests on this individual. The bactericidal activity of this serum in the two later tests was markedly lower, more probably because of lessened activity of the complement than of decreased titer of antibodies, so that the average index of the dilutions up to and including 1:1,280 is low relative to that of the two higher dilutions. At these times of testing and at monthly intervals between, the serum of this animal regularly showed partial agglutination of *Br. abortus* in a dilution of 1:100.

Just how much difference in antibacterial action toward these two strains is required in order to be able to state definitely that there is significant differential action of the serum can hardly be answered. However, even small differences in differential activity, if repeatable over a series of tests, would certainly be considered significant. These tests should be considered on that basis.

The serum of this animal (15E) did not differ in the presence or absence of bactericidal activity toward the two strains, but rather in the number of bacteria of each strain acted upon at the respective dilutions. The general statement can be made in respect to the serum of this and all the vaccinated animals, as was done above for the serum of normal animals, that wherever there was a difference in antibacterial activity, it was practically always in the direction of greater activity toward strain 19.

The serums of the 11 other animals, listed in table 2, gave antibacterial activity comparable in general with the serum dilutions of 15E. Of those whose serums were tested at a dilution of 1:10,240, only two showed reactivity, viz, 2g and 11g. The serums of 20E, 1F, 63F, 6g, 25g, 33g, and 38g were reactive at dilutions of either 1:5,120 or 1:2,560, while the serums of 8g and 63g were reactive at a dilution of 1:1,280, and only slightly if at all, at 1:2,560. The highest agglutination titer at the time of these tests was a partial reaction at 1:200 for the serum of 6g. The lowest titers (partial reactions at 1:25 or 1:50) of agglutinins were displayed at the time of the tests by the serums of 1F, 2g, 11g, 33g, and 63g, while those of the others were partially reactive at dilutions of 1:100. There appears to be no significant correlation here between the titers of agglutinins and bactericidins.

From these results it may be stated that the serum of vaccinated animals with agglutinating titers at or below those defining "reactors," as shown by these data, nevertheless may have an appreciable titer of bactericidins for *Br. abortus* of either high or low virulence. These results substantiate those previously reported from this laboratory (9), which showed the presence of bactericidins in appreciable titer in

the serums of animals artificially exposed to a virulent strain of *Br. abortus* (1, 2) and from whose serums agglutinating antibodies had disappeared. Although the average indices of bactericidal activity of the serum and serum dilutions of these animals did not *always* show a differential reactivity toward strain 19 and a virulent strain of *Br. abortus*, differential reactivity was shown in the *majority* of the dilutions of the serums of *all* the animals. These results show a high negative correlation between the bactericidal activity of the serum of these vaccinated animals and the degree of virulence of the organism. They also show that, even in serums from which agglutinins have disappeared, other antibodies for the same organism may be present. Perhaps these latter have more significance in the resistance of an animal than do the agglutinating antibodies.

Within the period in which these tests were made (i. e., 6 to 18 months after vaccination) there was no definite indication of a lowering of the bactericidal titer. That is, about an equal number of serums showed no change in titer as compared with the number which showed a possible reduction in activity. It is not implied, however, that the bactericidal titer of the serum of any of these would remain fixed.

Parallel studies to those reported in this paper are in progress at present on the serums of animals vaccinated as calves with strain 19, having as one objective to test the duration of the immunity induced by vaccination. The serums of these thirty-odd animals have likewise exhibited a differential activity toward strain 19 and a virulent strain of *Br. abortus*. The details of these studies will be reported later.

SUMMARY

The bactericidal action of the serum from both normal and vaccinated cattle has been shown to depend on the combined activity of antibody and complement.

The serum of normal animals usually has an appreciable bactericidal activity at dilutions of 1:40 or 1:80, that of some individuals at 1:160, rarely higher. The serum of animals vaccinated as adults, or nearly so, showed a definite antibacterial activity to *Brucella abortus* at dilutions of 1:1,280 and even at 1:10,240 in some individuals. The serum of these individuals showed partial agglutinating reactions no higher than 1:100, but with that from one cow at 1:200.

In the majority of the tests, undiluted or diluted serum destroyed more organisms of strain 19 than of the more virulent strain.

These tests show that even in the almost complete absence of agglutinating antibodies the serum of a vaccinated animal may exert antibacterial activity against *Br. abortus* greater than that of the serum of normal animals. These results substantiate a previous report on a parallel finding in the serum of animals once infected, but with a titer of agglutinating antibodies no higher than in normal cattle.

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ANALYSIS OF FIRE SPREAD IN LIGHT FOREST FUELS¹

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INTRODUCTION

On the national forests there has been a trend during recent years toward placing on a systematic basis such fire-control practices as rating fire danger, determining the proper size of suppression crews and speed of attack, and planning fire-suppression strategy. This trend has resulted in greatly improving the techniques of planning and managing the fire-control organization. At the same time it has revealed a serious lack of essential information. The outstanding need is for specific data on the rate at which forest fires may be expected to spread under various conditions of forest cover, weather, and topography.

Earlier studies in California by Show (13)⁴ and Curry and Fons (6, 7) consisted of observations on the rate of fire spread under natural forest conditions of fuel, weather, and topography. The initial series of experiments by Curry and Fons (6) in one of the least complex fuel types, ponderosa pine needles, provided data on the empirical relationship between the rate of fire-perimeter increase, litter moisture content, wind velocity, slope, and time from origin of fire. Similar observations were made on other fuel types.

Analysis of these data made it apparent that without a basic understanding of the many physical processes involved, the results of the experiments could not be applied beyond the limits of the conditions under which the observations were made. Therefore, it became necessary to determine the fundamental laws governing the rate of fire spread in forest-type fuels. Under field conditions, however, none of the important factors, such as the attributes of the atmosphere, the arrangement of the fuel bed, and the physical properties of the fuel particles, remained sufficiently uniform throughout an experiment to allow exact description of the numerous variables influencing the rate of fire spread. In order to understand the influence of these variables it was decided to conduct experiments with model fires in beds of chosen homogeneous fuel particles. These were at first performed by Curry and Fons (7) in still air for different conditions and kinds of fuel, fuel-moisture content, and fuel-bed compactness, and later by Fons (9) in a wind tunnel in which air velocity was controlled.

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⁴ Italic numbers in parentheses refer to Literature Cited, p. 121.

Concurrently with the latter experiments, an analytical treatment of the rate of spread of fire was being developed and utilized to direct the course and improve the techniques of fire control. This experiment and its results are described in this paper.⁵

GLOSSARY OF SYMBOLS

A =cross-sectional area of fuel particle, sq. in.

A_s =surface area of particle, sq. in.

a, b, c, \dots, n =subscripts designating different fuels.

$$B = \frac{W_a}{A_a \gamma_a} + \frac{W_b}{A_b \gamma_b} + \frac{W_c}{A_c \gamma_c} + \dots + \frac{W_n}{A_n \gamma_n}$$

C =proportionality constant relating a particular kind of fuel bed to a standard chosen bed.

C_p =specific heat of dry fuel at constant pressure, B. t. u./lb. ° F.

\bar{C}_p =specific heat of moist fuel at constant pressure, B. t. u./lb. ° F.

C_t =temperature-difference ratio coefficient.

D =diameter of fuel particle, inches.

d =prefix indicating differential, also depth of fuel bed, inches.

E =fuel particle shape factor, $\sigma A^{1/2}$.

f =over-all transfer factor, also subscript indicating flame or film.

f_c =film conductance for convection, and conduction, B. t. u./sq. ft. hr. ° F.

f_r =heat transfer factor for radiation, B. t. u./sq. ft. hr. ° F.

h =heat of adsorption of dry fuel, B. t. u./lb.

k_f =thermal conductivity of the air film, B. t. u./sq. ft. hr. ° F./ft.

L =spacing between particles, inches.

M =moisture content, weight of water/weight of dry fuel.

N =number of particles.

q =total rate of heat transferred, B. t. u./hr.

q_c =rate of heat transferred by convection and conduction, B. t. u./hr.

q_r =rate of heat transferred by radiation, B. t. u./hr.

R =rate of spread or $\frac{dr}{dt}$ in r -direction, ft./hr.

S =total fuel surface in volume V_1 , sq. in.

T =temperature, degrees Rankine.

t =temperature of fuel particle at time θ , ° F.

t_0 =temperature of fuel particle far away from flame, ° F.

t_1 =temperature of fuel particle near flame, ° F.

t_f =temperature of flame, ° F.

t_i =ignition temperature of fuel particle, ° F.

V =wind velocity at 1 foot above fuel-bed surface, m. p. h.

V_1 =volume of fuel bed, cu. in.

V_2 =volume of fuel in fuel bed volume V_1 , cu. in.

v =volume of particle, cu. in.

W =weight of dry fuel, lbs.

γ =(gamma) density of dry fuel, lbs./cu. ft.

$\bar{\gamma}$ =(gamma bar) density of moist fuel, lbs./cu. ft.

ϵ =(epsilon) flame emissivity.

θ =(theta) time in hours.

θ_i =ignition time of fuel; time in hours to raise a fuel particle from its initial temperature to ignition temperature.

λ =(lambda) volume of voids per unit of fuel surface, inches.

ν_f =(nu) kinematic viscosity of the film, sq. ft./hr.

σ =(sigma) surface-volume ratio, or fineness of fuel, inches⁻¹ (1÷number of inches).

ϕ =(phi) slope of terrain, percent.

⁵ A portion of the analysis in its earlier stages was submitted by the author in May 1940 as a thesis, entitled "Analytical Considerations of Model Forest Fires," in partial fulfillment of the requirements for the degree of master of science in the Department of Mechanical Engineering of the University of California.

DERIVATION OF EQUATIONS FOR RATE OF SPREAD IN AN IDEALIZED HOMOGENEOUS FUEL BED

Forest cover may be regarded as a heterogeneous mixture of dead and green fuel particles of varying size, shape, and density. Two layers may be distinguished: a lower layer of inflammable litter next to the ground surface, and an upper layer of dead and green standing vegetation. A fire may spread through either or both layers at a definite rate depending on the magnitude of many variables pertaining to (1) the atmosphere, (2) the fuel bed, (3) the fuel particles, and (4) the topography. The fire will spread quickest in that direction and through that layer where ignition is most favorable, or where resistance to flame propagation is at a minimum.

A forest fire characteristically travels more or less rapidly through the inflammable cover, singeing superficially only the finer, more ignitable particles, later slowly and thoroughly consuming the great mass of heavier fuels. In this theory of the rate of spread it is important to center our attention on the action at the head of the fire, where there is generally ample oxygen to support combustion. At

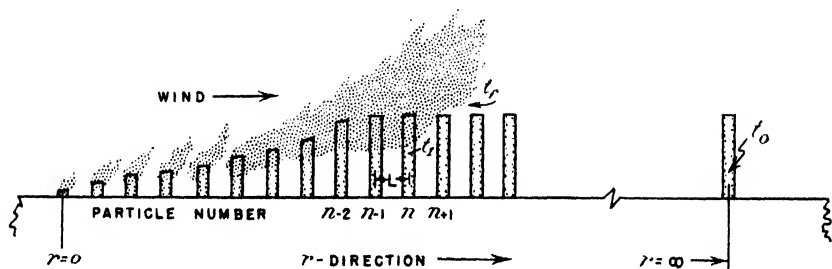


FIGURE 1.—Graphic representation of the rate of spread of fire in an idealized fuel bed composed of identical rodlike fuel particles.

this point only sufficient heat need be produced for a self-supporting fire to bring the immediate adjoining fuel to ignition temperature. Accordingly, flame propagation, or rate of fire spread in a fuel bed, may be visualized as proceeding by a series of successive ignitions, its magnitude controlled primarily by the ignition time of the particles and the distance between them.

As in other complex natural phenomena, the problem must be idealized in order to facilitate mathematical treatment of the theory. It is believed, however, that doubt as to the applicability to field conditions of the theoretical equations developed by this idealization will be removed when the predicted effects of the individual variables on rate of spread are compared with previous experimental results and with observations under actual field conditions.

RATE OF SPREAD AS A FUNCTION OF PARTICLE SPACING AND IGNITION TIME

For this derivation an idealized bed is assumed (fig. 1) composed of identical rodlike fuel particles, standing upright at equal distances from each other. If θ_i is the time for the n particle to burst into

flame after the $(n-1)$ particle has ignited, and if L is the distance between the particles, it follows that the rate of spread R is

$$R = \frac{L}{\theta_i} \quad (1)$$

where $R = \frac{dr}{d\theta}$ in the r -direction down the row of fuel particles.

RATE OF SPREAD AS A FUNCTION OF FUNDAMENTAL VARIABLES

The ignition time θ_i in equation (1) depends on two types of variables: Those dealing with (a) the fuel particle and (b) the atmosphere surrounding the particle.

Again it is assumed that the bed is composed of idealized fuel particles (fig. 1) spaced at a uniform distance L and that immediately upon ignition of the $(n-1)$ particle its flame at temperature t_f extends far enough to be in contact with the n particle which is at a temperature t_i . When the latter particle is exposed to the gases of flame temperature, t_f , it will absorb heat by convection, conduction, and radiation.

In the theory of fluid flow (11, 14) it is generally accepted that when a gas or liquid moves over a solid a thin film of fluid forms on the surface. This becomes thinner as the velocity of the fluid increases, and the thinner the film the more rapid will be the transfer of heat to the solid by conduction, convection, and radiation. The film conductance or transfer factor, f , is used to express the net rate of heat transfer through the film per unit area per degree difference in temperature. Factor f is the sum of two transfer factors, f_c for conduction and convection, and f_r for radiation. These are determined independently because the heat exchange by radiation depends primarily on the temperatures of the surfaces, while that for conduction and convection depends on variables such as the physical properties of the fluid, the shape of the solid surface, and the velocity of the fluid past the solid boundary. The method of calculating the transfer factors is discussed below.

The heat transferred by convection and conduction in unit time from the atmosphere at t_f to the particle is

$$q_c = f_c A_s (t_f - t) \quad (2)$$

The heat transferred by radiation in unit time from the atmosphere at t_f to the particle is approximately:

$$q_r = f_r A_s (t_f - t) \quad (3)$$

The total heat absorbed in unit time is

$$q = q_c + q_r = (f_c + f_r) (t_f - t) A_s \quad (4)$$

where

A_s = surface area of the particle;

t_f = uniform temperature of flame surrounding the particle;

t = temperature of particle at any time θ , assumed to be uniform throughout the volume of the particle, since the temperature gradient is very low for fine fuel particles with large surface-volume ratio;

f_c and f_r are the coefficients of heat transfer for convection and radiation energy, respectively.

The rate of heat absorption, q , will cause a rate of temperature change $\frac{dt}{d\theta}$, according to the density of the moist particle, $\bar{\gamma}$, the specific heat of the moist particle, \bar{C}_p , and the volume of the fuel particle, v , as follows:

$$q = \bar{\gamma} \bar{C}_p v \frac{dt}{d\theta} \quad (5)$$

Substituting (5) in (4) yields:

$$\frac{dt}{(t_f - t)} = \frac{(f_c + f_r) A_s}{\bar{\gamma} \bar{C}_p v} d\theta \quad (6)$$

By integrating equation (6), with $\frac{(f_c + f_r) A_s}{\bar{\gamma} \bar{C}_p v}$ assumed constant, equation (7) is obtained:

$$-\ln(t_f - t) = \frac{(f_c + f_r) A_s \theta}{\bar{\gamma} \bar{C}_p v} + \ln C \quad (7)$$

where \ln represents the Napierian logarithm.

The initial conditions to be satisfied are: when $\theta = 0$, $t = t_i$. By substituting these values in (7) the constant of integration becomes

$$\ln C = -\ln(t_f - t_i) \quad (8)$$

Substituting (8) in (7) and rearranging

$$\ln \left(\frac{t_f - t_i}{t_f - t} \right) = \frac{(f_c + f_r) \sigma \theta}{\bar{\gamma} \bar{C}_p} \quad (9)$$

where $\frac{\sigma A_s}{v} =$ the surface-volume ratio of fuel particle.

The final conditions to be satisfied are: when $\theta = \theta_i$, $t = t_i$, where $\theta_i =$ ignition time, and $t_i =$ ignition temperature.

Making these substitutions and rearranging for θ_i , equation (9) becomes

$$\theta_i = \frac{\bar{\gamma} \bar{C}_p}{\sigma (f_c + f_r)} \ln \left(\frac{t_f - t_i}{t_f - t_i} \right) \quad (10)$$

Equation (10) is an expression for the ignition time, θ_i , in terms of the physical properties of the light fuel particle,⁶ the flame temperature, and the heat transfer factors.

The rate of spread for an idealized homogeneous fuel bed is derived directly by substituting the equivalent of θ_i from equation (10) in equation (1):

$$R = \frac{(f_c + f_r) \sigma L}{\bar{\gamma} \bar{C}_p \ln \left(\frac{t_f - t_i}{t_f - t_i} \right)} \quad (11)$$

In this equation R is expressed in terms of certain fundamental variables that control the spread of a fire in light forest fuels. Such

⁶ Derivation of an expression for heavier fuel particles, in which there is an appreciable temperature gradient through the particle, is possible by the application of an appropriate form of the conduction equations (5).

quantities as wind velocity, moisture content, time, and topography, which are ordinarily regarded as controlling spread, do not appear in the equation. They affect the rate of spread indirectly through their influence on the more fundamental variables.

FILM CONDUCTANCE FOR CONVECTION

Experimental data for heat transfer from gases to single cylindrical rods, with the gas flowing at right angles to the axis, have been collected by numerous investigators. Data for cylinders from 0.0004 to 3.75 inches in diameter (11, p. 218), applicable to fine forest fuels such as needles and twigs, have been summarized and correlated in terms of dimensionless quantities related by the following empirical equation, which may be used as an approximation for calculating f_c :

$$\frac{f_c D}{k_f} = 0.45 + 0.33 \left(\frac{VD}{\nu_f} \right)^{0.56} \quad (12)$$

where f_c = film conductance for forced convection

D = diameter of cylinder

k_f = thermal conductivity of the air film

ν_f = kinematic viscosity of the air film

V = air velocity.

Thermal conductivity and kinematic viscosity depend on the arithmetic mean temperature of the film. Values of these properties for air are given in the International Critical Tables for a wide range of temperatures.

Forest fuels such as needles and twigs are nearly enough cylindrical in shape to allow replacing D in equation (12) with $\frac{4}{\sigma}$, where σ is the surface-volume ratio of the fuel particle.

HEAT TRANSFER COEFFICIENT FOR RADIATION AND FLAME TEMPERATURE

The net rate of heat transfer by radiation, q_r , to a small particle surrounded by a flame (14) is obtained by the following equation:

$$q_r = 0.172 \epsilon A_s \left[\left(\frac{T_f}{100} \right)^4 - \left(\frac{T_1}{100} \right)^4 \right] \quad (13)$$

where ϵ = flame emissivity ⁷

T_f = flame temperature

T_1 = particle temperature

A_s = surface area of the particle

An expression for f_r may be derived by substituting simplified equation (3) in general equation (13),

$$f_r = \frac{0.172 \epsilon \left[\left(\frac{T_f}{100} \right)^4 - \left(\frac{T_1}{100} \right)^4 \right]}{t_1 - t_i} \quad (14)$$

⁷ Radiant emissivity is defined as the ratio of the total emissive power (total radiant energy emitted per unit time from unit surface) of a surface to the total emissive power of a black body at the same temperature.

Flame temperatures were measured by chromel-alumel thermocouples held at several points in the luminous flames of different kinds of burning forest fuel. The average temperature so measured was approximately 1500° F. Simultaneously, the emissive power of the flames was measured by a portable radiometer described by Boelter (2). From the measured flame temperature, the radiometer element temperature, and the flame emissive power, q_r , the emissivity, ϵ , was calculated by the radiation equation (13). The approximate value of ϵ so determined was 0.227.

Until more data are acquired, the values used for ϵ and t_f in calculating f_r are 0.23 and 1500° F., respectively. Since the particle is being heated until it reaches ignition temperature, an approximation must be made for the fuel temperature, t_i . On the assumption that the rate of change of the particle temperature is constant, the fuel temperature, t_i , may be approximated by using the arithmetic mean of the ignition temperature, t_b , and the fuel temperature, t_0 . While t_i may vary over a considerable range between t_i and t_0 , its approximation at the mean value of these two will not result in an appreciable error in the calculation of f_r .

IGNITION TEMPERATURE

There is no general agreement as to the ignition temperatures of solid fuels. The values determined by several investigators whose work was reviewed by Brown (3) show a large variation. For instance, values as high as 1200° and as low as 650° F. have been obtained for sawdust and as low as 410° for wood shavings. The principal causes of the variations are (1) the definition of ignition temperature, which may refer to the specimen temperature or to the external temperature of a bar, an ambient gas, or an oven, and (2) the criteria chosen by different investigators to indicate the ignition point. Criteria that have been used are the appearance of glow, the appearance of flame, or some critical point in a temperature- or pressure-time curve.

To be consistent with the preceding theory and the equations developed for the rate of fire spread in light forest fuels, the following definition is proposed: The ignition temperature of a light forest-fuel particle surrounded by ambient hot gases is the average specimen temperature at the instant a flame appears. For light forest fuels of known physical properties exposed to hot gases of known temperature, the average specimen temperature may be calculated by equation (10) from the ignition time, which is determined experimentally. Until such experimental data are available, an arbitrary chosen value of 550° F. is recommended for all light forest fuels irrespective of fuel size and moisture content. Jones and Scott⁸ found the ignition temperature of dry wood to be in the range of 518° to 554° F.

PARTICLE SPACING AND SURFACE-VOLUME RATIO

To calculate the rate of fire spread in a fuel bed by equation (11), L , the spacing, must be related to the measurable fuel and fuel-bed variables.

⁸ JONES, G. W., and SCOTT, G. S. CHEMICAL CONSIDERATIONS RELATING TO FIRES IN ANTHRACITE REFUSE. U. S. Bur. Mines Rpt. Invest. 3468, 13 pp., illus. 1939. [Processed.]

For a bed of fuel, the ratio of voids to fuel-surface area is

$$\lambda = \frac{V_1 - V_2}{S} \quad (15)$$

where λ = volume of voids per fuel-surface area

V_1 = volume of fuel bed

V_2 = volume occupied by the fuel in volume V_1

S = total surface area of fuel in volume V_1 .

The reciprocal of λ may be thought of as the compactness of the fuel.

The volume of the idealized fuel bed is

$$V_1 = L^2 d N \quad (16)$$

where L = spacing of particles

d = depth of fuel bed

N = total number of particles.

The volume occupied by the fuel in the bed of volume V_1 is

$$V_2 = \frac{W}{\gamma} \quad (17)$$

where W is the total weight and γ is the density of the dry fuel. The total surface area of the fuel in bed of volume V_1 is

$$S = \frac{\sigma W}{\gamma} \quad (18)$$

Substituting equations (16), (17), and (18) in (15) gives

$$\lambda = \frac{L^2 d N - W/\gamma}{\sigma W/\gamma} \quad (19)$$

Solving equation (19) for L and letting the cross-sectional area $A = \frac{W}{N\gamma d}$ (since for an idealized bed the length of the particles is equal to the depth of the bed) (19) reduces to:

$$L = A^{1/2} (1 + \sigma\lambda)^{1/2} \quad (20)$$

Equation (20) expresses the particle spacing in terms of the cross-sectional area, the surface-volume ratio of the fuel particle, and the ratio of voids to fuel-surface area of the bed.

Multiplying both sides of equation (20) by σ and letting $E = \sigma A^{1/2}$ then

$$\sigma L = E(1 + \sigma\lambda)^{1/2} \quad (21)$$

The quantity E is independent of the size of the fuel particle and depends only on the shape of the cross section. For instance, for round sticks regardless of size, $E = 2\pi^{1/2}$; for square sticks, $E = 4.0$; for more irregular cross sections, $E > 4.0$. The cross-sectional area of an irregularly shaped particle may be determined from the relation

$A = \frac{p^2}{4}$, where p is the perimeter and σ the surface-volume ratio.

SPECIFIC HEAT

Since the fuel particles nearly always carry adsorbed water, the specific heat of the moist fuel, \overline{C}_p , may be expressed in terms of specific heat of dry fuel, its moisture content, and the heat of adsorption.

The total heat required to raise a moist fuel particle at temperature t_0 to ignition temperature t_i is

$$H = (W + MW)\overline{C}_p(t_i - t_0) \quad (22)$$

where W = dry weight of fuel

M = moisture content, weight of water per unit weight of dry fuel

\overline{C}_p = specific heat of moist fuel.

The total heat, H , has three components: the heat required (1) to separate the water from the fuel, that is, the heat of adsorption; (2) to raise the dry fuel from temperature t_0 to ignition temperature, t_i ; and (3) to vaporize the water and superheat the vapor to temperature t_i .

The heat required to raise the dry fuel to ignition temperature is

$$H_1 = WC_p(t_i - t_0) \quad (23)$$

where C_p is specific heat of dry fuels (8)⁹;

and to raise the water to temperature t_i is

$$H_2 = MW(h_i - h_0)$$

where $h_i = 1,057 + 0.46t_i$, the heat (1, p. 309) of the superheated steam at low pressure in B. t. u. per pound, and $h_0 = t_0 - 32$, the heat content

of water at temperature t_0 , whence

$$H_2 = MW(1,089 + 0.46t_i - t_0) \quad (24)$$

The heat of adsorption per unit dry weight of fuel is h , and heat required for weight of dry fuel W is

$$H_3 = Wh \quad (25)$$

The heat of adsorption for wood at 32° F. is approximately 32 B. t. u. per pound of dry fuel (10, p. 286).

Adding equations (23), (24), and (25), the total heat, H , to bring W , weight of moist fuel, to ignition temperature t_i is

$$H = WC_p(t_i - t_0) + MW(1,089 + 0.46t_i - t_0) + Wh \quad (26)$$

⁹ The equation for specific heat of dry wood from results considered as preliminary is

$$C_p = 0.246 + 0.00063 \left(\frac{t_i + t_0}{2} \right) \quad \text{B.t.u./lb. } ^\circ\text{F.}$$

fire" and the amount of fuel burning at the head of the fire. Temperature t_1 varies with the flame angle because, as the angle becomes smaller, more hot gases pass directly over the unburned fuel bed in the direction of the main flame front; thus the fuel particles take on more heat by convection and radiation. Temperature t_1 varies with the size of flame, which governs the amount of heat transferred to the unburned fuel particles by radiation. The time from start of fire also affects temperature t_1 , because at zero time t_1 must equal t_0 , but at subsequent intervals the fuel particles ahead of the fire have taken on heat, causing t_1 to become greater than t_0 . Temperature t_1 is not easily measured even under ideal conditions. Temperature t_0 of fuel far removed from the advancing flame front is, however, readily determined. Temperature t_1 may be eliminated by substituting t_0 for it and correcting this new temperature-difference ratio in equation (11) by a coefficient, C_t , in the following manner:

$$\text{Let} \quad C_t \left(\frac{t_f - t_0}{t_f - t_1} \right) = \frac{t_f - t_1}{t_f - t_0} \quad (28)$$

$$\text{wherefore} \quad C_t = \frac{t_f - t_1}{t_f - t_0} \quad (29)$$

Figure 2 shows diagrammatically the fuel-temperature gradient and temperature differences at and near the flame front. For all practical forest conditions t_f may be assumed to be about 1500° F. The effect of increasing wind or slope makes t_1 numerically larger, which makes C_t smaller, according to equation (29). Since t_1 increases numerically with time from start of fire and size of flame, these also will make C_t smaller. Any independent change in t_0 will be nearly compensated by a change in the same direction in t_1 and will not therefore greatly change C_t . The temperature-difference ratio coefficient is primarily a function of wind velocity, slope, size of flame, and time from start of fire and must be evaluated experimentally. The approximate numerical range of C_t may be determined by substituting a reasonable value of t_0 and the limiting values of t_1 in equation (29). For the field conditions of $t_0 = 80^\circ \text{ F.}$ and $t_1 = 550^\circ \text{ F.}$, C_t will vary from 0.67 to more than 1.00. At the very start of a fire, when $t_1 = t_0$, $C_t = 1.00$. For the extreme conditions of zero flame angle, which occur at extremely high wind velocity or infinite slope, C_t will approach 0.67 when t_1 approaches t_f . For fires burning in still air in dead fuel exposed to full sunlight, C_t may become somewhat greater than 1.00 with time from start of fire; for under these conditions t_0 will be high and the fuel may actually be cooled by the indraft.

TRANSFORMATION OF EQUATIONS DERIVED FOR IDEALIZED HOMOGENEOUS BEDS TO NATURAL HOMOGENEOUS BEDS

In the idealized fuel bed all particles were assumed to be vertically oriented with equal spacing. In the natural homogenous bed, such as a mass of fallen pine needles, more or less horizontally oriented at random, the spacing is not equal but has instead voids of varying sizes. There are other differences between idealized and natural beds. In the latter the particles are in occasional contact with one another, and will not always be oriented with the flow of hot gases as in the idealized bed; this will influence the rate of heat conducted into

the fuel. There may also be a fuel-moisture gradient in the natural fuel bed.

Granting that all the variables affecting the rate of spread have been included in the derivation, according to the theory of similitude (12, p. 78), if the processes of burning in the two beds follow the same laws and the respective variables stand in a constant ratio to each other over their whole range, the application of equation (11), derived for idealized beds, to fires in natural beds is permissible. On the assumption that the individual variables in the two processes are respectively proportional, as required by the theory of similitude, equations relating these variables may be written; for example, the relation between spacing L_1 of the idealized and L_2 of the natural bed may be expressed as

$$L_1 = C_L L_2$$

where C_L is a constant quantity for the whole range of spacing. Other variables such as specific heat, density, etc., may be dealt with in a similar manner.

Accordingly, the rate-of-spread equation will be identical in the natural and idealized beds, except that each variable will be multiplied

by its corresponding coefficient. With $\frac{t_f - t_i}{t_f - t_i}$ replaced by $C_t \left(\frac{t_f - t_0}{t_f - t_i} \right)$,

in which C_t serves as both the temperature-difference ratio coefficient and as a coefficient relating the natural to the idealized values of t_f and t_0 , equation (11) for a natural fuel bed becomes

$$R = \frac{C_f(f_c + f_r)C_\sigma\sigma C_L L}{C_{\gamma}\bar{\gamma}CC_p\bar{C}_p \ln \left[C_t \left(\frac{t_f - t_0}{t_f - t_i} \right) \right]} \quad (30)$$

Inasmuch as the coefficients C_f , C_L , etc., remain constant

$$\text{let} \quad C = \frac{C_f C_\sigma C_L}{C_{\gamma} C_p} \quad (31)$$

then

$$R = \frac{C(f_c + f_r)\sigma L}{\bar{\gamma}\bar{C}_p \ln \left[C_t \left(\frac{t_f - t_0}{t_f - t_i} \right) \right]} \quad (32)$$

where C represents a proportionality constant relating the rate in a natural bed with the rate in a standard bed under identical conditions.

The coefficients C_t and C in equation (32) are evaluated experimentally. As shown in the above discussion, C_t is primarily a function of wind velocity, slope, size of flame, and time from start of fire. The numerical value of C is dependent in part on the type of fuel bed, that is, whether it is a mass of fallen litter or standing vegetation, such as grass or brush, and in part on the technique used in measuring the variables, such as wind, fuel moisture, and fuel temperature. To determine the numerical value of C_t for any particular wind, time, and slope it is first necessary to evaluate C for the particular fuel used in the experiments. However, in order to obtain the value of C with reasonable accuracy we must have data for a wide range of fuel temperature, and this is experimentally impracticable. On the other hand, if C_t be evaluated with C taken as unity for a

chosen standard natural fuel bed, for which a precise technique for measuring the wind, moisture content, and other variables has been developed (to a point where measurements of all variables can be duplicated), then C can be relatively evaluated for any other kind of natural bed. This is done by determining R experimentally for a particular kind of bed and computing a value for C by equation (32), using C_i as evaluated for the standard bed. The coefficient C determined in this manner would then be a constant, relating the particular kind of natural bed not with the idealized bed, but with the chosen natural standard.

DERIVATION OF EQUATIONS FOR RATE OF SPREAD IN NATURAL HETEROGENEOUS FUEL BEDS

Fuel beds of the homogeneous type are of rare occurrence in nature. Normally a natural bed is composed of particles varying in size, shape, density, and specific heat. Since the ignition time, ϕ_i is dependent on the material and dimensions of the particles, it follows that the rate of spread will be different for different particles.

Consider an idealized heterogeneous bed composed of particle types a, b, c, \dots, n , where all particles are of the same length, equal to the depth of the fuel bed, and are evenly spaced at distance L . If $N_a, N_b, N_c, \dots, N_n$ are the number of particles of respective fuel types per unit area, and N is the total number of particles, then in a given length of bed each kind will be present in amount propor-

tional to $\frac{N_a}{N}, \frac{N_b}{N}, \frac{N_c}{N}, \dots, \frac{N_n}{N}$. If these fractions are represented as $X_a, X_b, X_c, \dots, X_n$ and if $R_a, R_b, R_c, \dots, R_n$ represent the respective rates of spread through the different fuel types, the gross rate of spread of the mixture will be ¹⁰

$$R = \frac{1}{\frac{X_a}{R_a} + \frac{X_b}{R_b} + \frac{X_c}{R_c} + \dots + \frac{X_n}{R_n}} \quad (33)$$

¹⁰ Equation (33) is analogous to one for total conductance of a series electrical circuit of length L composed of several electrical conductors of length l , each of different conductance but occurring n times.

For example, let $N_a, N_b, N_c, \dots, N_n$ be the number of times conductors of length l , having conductances $C_a, C_b, C_c, \dots, C_n$, respectively, occur. The total length of the circuit will be $L = N_al + N_bl + N_cl + \dots + N_nl$,

and the total conductance

$$C = \frac{N_a + N_b + N_c + \dots + N_n}{\frac{N_a}{C_a} + \frac{N_b}{C_b} + \frac{N_c}{C_c} + \dots + \frac{N_n}{C_n}}$$

Then let

$$X_a = \frac{N_a}{N_a + N_b + N_c + \dots + N_n}, X_b = \frac{N_b}{N_a + N_b + N_c + \dots + N_n}, \text{ etc.}$$

where $X_a, X_b, X_c, \dots, X_n$ are the fraction of the total conductance contributed by conductors type a, b, c, \dots, n respectively

Then

$$C = \frac{1}{\frac{X_a}{C_a} + \frac{X_b}{C_b} + \frac{X_c}{C_c} + \dots + \frac{X_n}{C_n}}$$

Let $W_a, W_b, W_c, \dots, W_n$ be the total dry weight of the several particles per unit area of the fuel bed. Then

$$N_a = \frac{W_a}{A_a \gamma_a d}, \quad N_b = \frac{W_b}{A_b \gamma_b d}, \text{ etc.} \quad (34)$$

where A 's and γ 's are the cross-sectional areas and the densities of the dry particles, respectively, and d is their length.

$$\text{By definition} \quad X_a = \frac{N_a}{N}, \quad X_b = \frac{N_b}{N}, \text{ etc.} \quad (35)$$

$$\text{and} \quad N = N_a + N_b + N_c + \dots + N_n \quad (36)$$

Equation (35) becomes, after substituting (34) and (36):

$$X_a = \frac{\frac{W_a/A_a \gamma_a}{\frac{W_a}{A_a \gamma_a} + \frac{W_b}{A_b \gamma_b} + \frac{W_c}{A_c \gamma_c} + \dots + \frac{W_n}{A_n \gamma_n}}}, \text{ etc.,} \quad (37)$$

for X_b, X_c, \dots, X_n .

Substituting equation (37) in (33) and letting

$$B = \frac{W_a}{A_a \gamma_a} + \frac{W_b}{A_b \gamma_b} + \frac{W_c}{A_c \gamma_c} + \dots + \frac{W_n}{A_n \gamma_n} \quad (38)$$

the following equation results:

$$R = \frac{B}{\frac{W_a}{A_a \gamma_a R_a} + \frac{W_b}{A_b \gamma_b R_b} + \frac{W_c}{A_c \gamma_c R_c} + \dots + \frac{W_n}{A_n \gamma_n R_n}} \quad (39)$$

Equation (39) is an expression for the rate of spread of fire through a heterogeneous bed in terms of fuel composition and the rates of spread through each component fuel. The rate for each component is calculated by equation (32) with the value of the compactness, $1/\lambda$, equal to that of the mixture.

To calculate the rate of fire spread in a heterogeneous bed by equation (39) the spacing, L , which is not directly measurable, must be determined from the fuel and fuel-bed characteristics. The measurable factors from which L may be computed are $V_1, V_2, W, \gamma, \sigma$, and λ , where λ is determined by equation (15).

For a given bed, the ratio of volume of voids to fuel-surface area is expressed by equation (15); the volume of a fuel bed by equation (16).

The volume occupied by the fuel in the heterogeneous bed of volume V_1 is $V_2 = V_a + V_b + V_c + \dots + V_n$ where V_a, V_b, \dots, V_n are volumes of the different kinds of particles. It follows that

$$V_2 = \frac{W_a}{\gamma_a} + \frac{W_b}{\gamma_b} + \frac{W_c}{\gamma_c} + \dots + \frac{W_n}{\gamma_n} \quad (40)$$

The total surface area of the fuel in this bed is

$$S = \frac{\sigma_a W_a}{\gamma_a} + \frac{\sigma_b W_b}{\gamma_b} + \frac{\sigma_c W_c}{\gamma_c} + \dots + \frac{\sigma_n W_n}{\gamma_n} \quad (41)$$

Substituting equations (16), (40), and (41), in (15) gives

$$\lambda = \frac{L^2 dN - \left(\frac{W_a}{\gamma_a} + \frac{W_b}{\gamma_b} + \frac{W_c}{\gamma_c} + \dots + \frac{W_n}{\gamma_n} \right)}{\frac{\sigma_a W_a}{\gamma_a} + \frac{\sigma_b W_b}{\gamma_b} + \frac{\sigma_c W_c}{\gamma_c} + \dots + \frac{\sigma_n W_n}{\gamma_n}} \quad (42)$$

Solving equation (42) for L^2 , then multiplying and dividing each term, the right side of the equation by the number of particles in that type of fuel, namely, $N_a, N_b, N_c, \dots, N_n$, letting $A_a, A_b, A_c, \dots, A_n$ equal $\frac{W_a}{\gamma_a N_a d}, \frac{W_b}{\gamma_b N_b d}, \frac{W_c}{\gamma_c N_c d}, \dots, \frac{W_n}{\gamma_n N_n d}$, and assuming a length of

fuel particles equal to the depth of the fuel bed, the following equation results:

$$L = [\lambda(\sigma_a A_a X_a + \sigma_b A_b X_b + \sigma_c A_c X_c + \dots + \sigma_n A_n X_n) + (A_a X_a + A_b X_b + A_c X_c + \dots + A_n X_n)]^{1/2} \quad (43)$$

where $X_a, X_b, X_c, \dots, X_n$ are defined by equation (37).

By substituting the equations for $X_a, X_b, X_c, \dots, X_n$ and simplifying, equation (43) becomes:

$$L = \left[\frac{\lambda}{B} \left(\frac{\sigma_a W_a}{\gamma_a} + \frac{\sigma_b W_b}{\gamma_b} + \frac{\sigma_c W_c}{\gamma_c} + \dots + \frac{\sigma_n W_n}{\gamma_n} \right) + \frac{1}{B} \left(\frac{W_a}{\gamma_a} + \frac{W_b}{\gamma_b} + \frac{W_c}{\gamma_c} + \dots + \frac{W_n}{\gamma_n} \right) \right]^{1/2} \quad (44)$$

where B is defined by equation (38).

Equation (44) is an expression for the particle spacing L for a heterogeneous fuel bed in terms of its composition. The assumption of fuel-particle length equal to depth of the fuel bed requires, of course, use of the coefficient (L in application of this measure to natural fuels. This coefficient is present in equation (32).

EXPERIMENTS IN NATURAL HOMOGENEOUS FUEL BEDS

EXPERIMENTAL PROCEDURE

For evaluating the coefficient C_i , ponderosa pine needles, being the most readily available uniform fuel in this region (California), were chosen as the natural fuel for the standard bed. In order to exercise control over all the variables, fires were studied on a model scale. Beds of needles 2 inches deep, 3 feet wide, and 8 or 12 feet long were prepared in trays with controlled compactness. These beds were placed in a wind tunnel (fig. 3) described elsewhere (9) and burned on the horizontal, i. e., at zero slope. Figure 4 shows the position of initial ignition of a fuel bed in the tunnel. Ignition was accomplished by an electrically heated coil placed on a match head inserted in the middle of a thin pyroxylin disk of 2-inch diameter. The fire traveling forward along the length of the bed was observed (fig. 5) from an overhead window. Each fire was allowed to burn

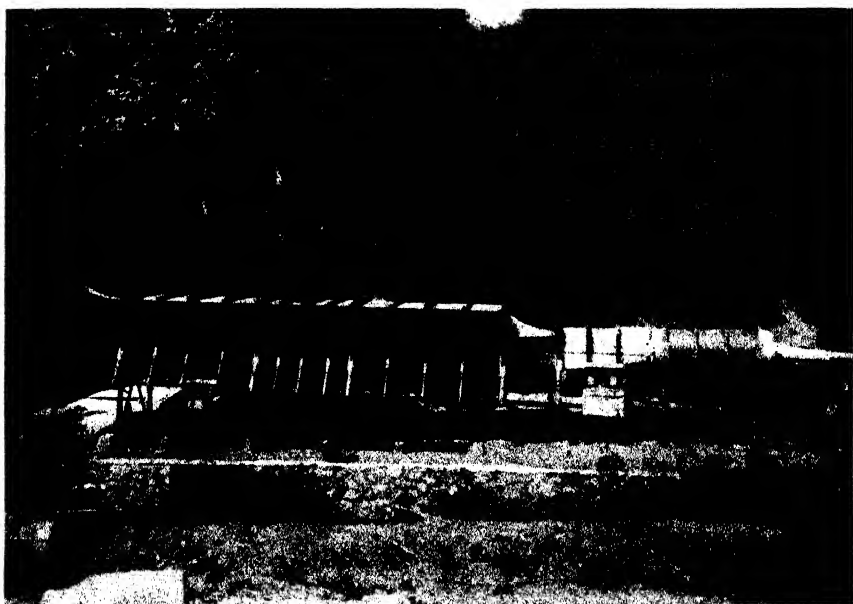


FIGURE 3.—Wind tunnel used in fire-behavior studies; test section is 30 feet long with a cross section 6 x 6 feet, over-all length 55 feet.

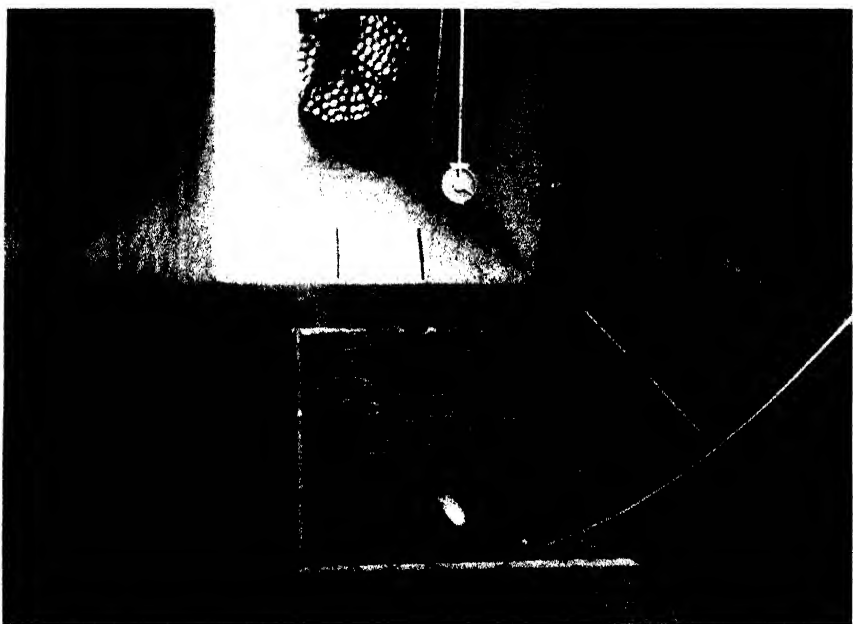


FIGURE 4.—Position of initial ignition of a fuel bed in the tunnel.

until it reached approximately 18 inches in width and was then instantly suppressed by water. A typical burned area is shown in figure 6.

The air velocity recorded for each fire was the velocity at 1 foot above the surface of the bed prior to ignition. A sample of fuel was collected at random from the surface of the bed just prior to burning and its moisture content was determined by xylene distillation (4). The fuel temperature was taken to be equal to the ambient air temperature, measured when the moisture sample was taken. Approx-



FIGURE 5.—Pine-needle fire 30 seconds after ignition, photographed from above; wind velocity 8 m. p. h.

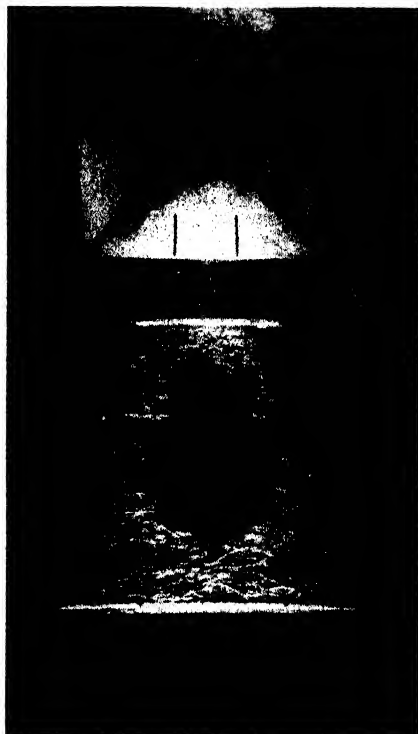


FIGURE 6.—Typical burned area of pine needles.

imately 200 fires were burned in fuel beds of ponderosa pine needles ($\sigma=128$ inches⁻¹) under varying conditions such as: wind velocity 2.0 to 12.0 m. p. h.; compactness, $1/\lambda$, 6.25 to 16.66 inches⁻¹; moisture content 4.0 to 15.0 percent; air temperature 50° to 85° F.

In order to simulate a standing type of fuel, such as brush, the beds were prepared by standing uniform twigs vertically and at equal spacing in sawdust treated with a fire-retardant chemical (fig. 7). The twigs were cut 7.5 inches in length from dead branches of ponderosa pine and segregated into three nominal size classes $\frac{1}{8}$, $\frac{3}{16}$, and $\frac{1}{4}$ inch in diameter. The bed was set afire by a 30-gm. bundle of pine needles and to avoid the influence of the burning needles, the twigs were allowed to burn a distance of about 4 feet before zero time was called.

The fire was not extinguished until it had reached the end of the bed, which was 12 feet in length (fig. 8). Observations were made and data taken only in that portion of the burn beyond the first narrow region influenced by the mode of ignition.

RESULTS

Numerical values of C_i were computed for each pine-needle fire by equation (32) using the observed rates of spread with C equal to unity.

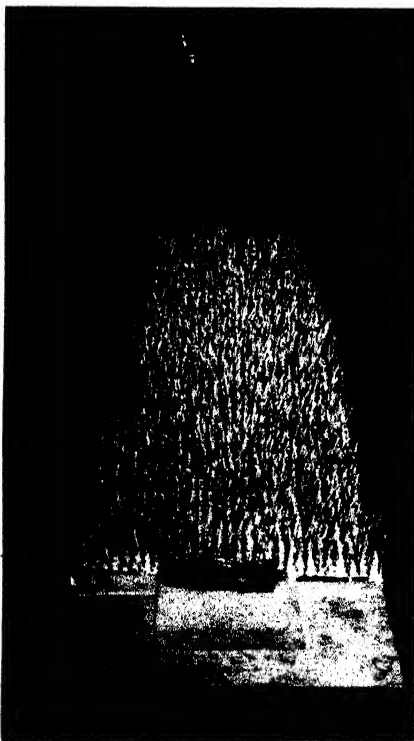


FIGURE 7.—Bed of ponderosa pine twigs $\frac{3}{16}$ inch in diameter with bundle of needles in the foreground used for ignition.



FIGURE 8.—Typical burned area of twigs.

The average values of C_i were computed for several wind classes and are shown in figure 9. A value for the rate of spread of each fire was calculated by using appropriate values of C_i from the curve in figure 9. Figure 10 shows the computed versus the actual rates of spread plotted to reveal the agreement. It will be noted that the points fall close to and evenly about a 45° line which represents agreement of calculated with experimental values.

The experimental data for the twig fires are presented in table 1, together with the calculated values for the coefficient C . The values of C for individual fires vary considerably from its mean of 1.61 owing mainly to the experimental technique used in igniting the twigs and to the error in judging the exact position of the fire at zero time and

the time when the fire reached the end of the bed. Therefore, the value of 1.6 for C , for this type of bed, must be considered as only approximate until more data have been obtained. Since C was assumed to be unity for the pine-needle bed, the value of 1.6 is in the right order of magnitude for a fuel bed with twigs standing vertically, inasmuch as it was assumed that the flow of the gases passing the particles in the standard pine-needle bed was at right angles. It would have been more nearly correct, however, to assume that the gas flow is at right angles for only half the particles, and parallel or

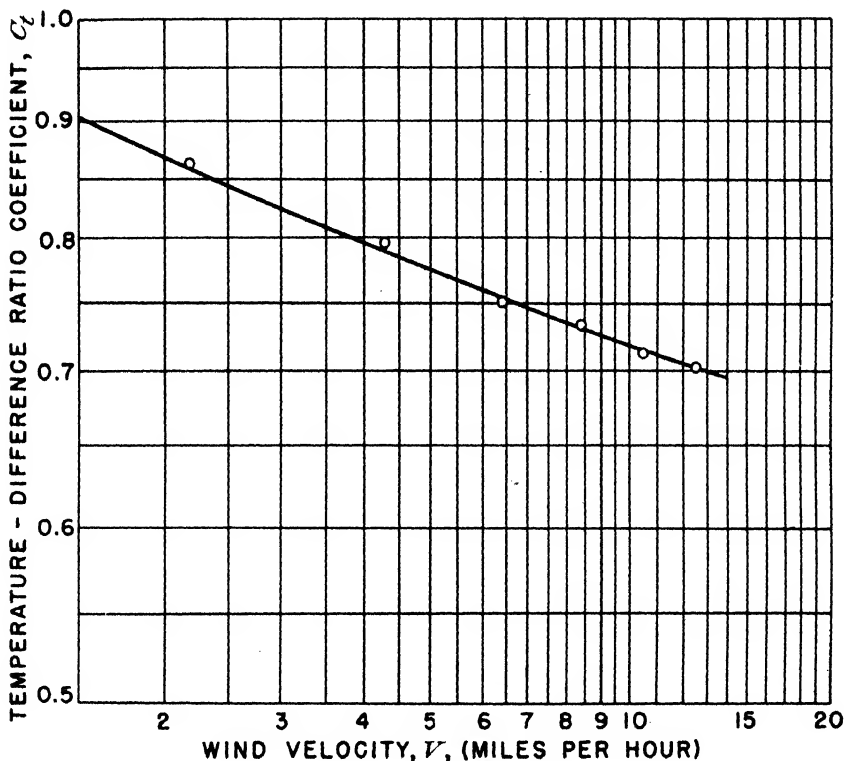


FIGURE 9.--Relation between temperature-ratio coefficient, C_t , and wind velocity, V (miles per hour) at 1 foot above fuel surface, zero slope, and 1 minute from start of fire. Fuel: Ponderosa pine needles.

longitudinal for the other half. Actually, in evaluating C_t by the type of bed chosen as standard, it might have been more proper to assume a value less than unity for C , in which case the numerical values of C_t would have been slightly higher. Then, with higher values of C_t for beds with twigs vertically oriented where cross flow is more nearly approached, C would have more nearly approached unity. Practically, however, since C may vary with the technique used in sampling fuel moisture and measurement of wind velocity, it would probably differ from unity for other types of fuel beds. Consequently, for the sake of simplicity it would be better to assume cross flow for all types of beds and let C , evaluated in that way, be a constant

relating a particular kind of natural bed with a standard pine-needle bed, for which f_c was calculated on the basis that the gases flow at right angles to the needles.

TABLE 1.—Experimental data and calculated values of C for model fires burning in fuel beds of ponderosa pine twigs placed vertically and spaced equally

Fire (number)	Experimental data						Calculations ¹				
	Wind velocity	Fuel temperature	Fuel moisture content	Fuel surface-volume ratio	Fuel spacing	Rate of spread	σL	$f_c + f_r$	$\frac{1}{\gamma C_p}$	$\ln \left[C_1 \left(\frac{t_f - t_o}{t_f - t_i} \right) \right]$	C
	(V)	(t_o)	(M)	(σ)	(L)	(R)					
	M p.h.	°F.	Pct.	Inches ⁻¹ (2)	Inches	Ft./hr.		B. t. u./ Sq. ft. hr. °F.	B. t. u./ Cu. ft. °F.		
22	4	80	12.4	20.7	1.50	195	31.0	14.7	24.6	0.180	1.89
3	4	86	9.1	20.7	1.50	223	31.0	14.7	22.2	.176	1.91
11	4	88	10.3	20.7	1.50	209	31.0	14.7	23.1	.174	1.84
51	6	69	11.9	15.7	1.50	225	23.6	15.5	23.9	.139	2.04
10	6	89	10.6	20.7	1.50	265	31.0	17.1	23.4	.125	1.46
21	6	79	12.1	20.7	1.50	285	31.0	17.1	24.3	.132	1.72
1	6	87	9.8	20.7	1.50	335	31.0	17.1	22.7	.126	1.81
37	6	68	11.4	27.8	1.25	367	34.8	19.0	23.5	.140	1.83
49	8	68	12.5	15.7	1.75	222	27.5	17.0	24.4	.105	1.22
56	8	73	10.9	15.7	1.75	264	27.5	17.0	23.3	.101	1.33
65	8	62	11.3	15.7	1.63	257	25.5	17.0	23.3	.109	1.51
40	8	71	10.7	15.7	1.50	294	23.6	17.0	23.1	.103	1.74
52	8	68	12.2	15.7	1.50	242	23.6	17.0	24.2	.105	1.53
39	8	71	10.6	15.7	1.38	306	21.6	17.0	23.0	.103	1.97
48	8	61	11.4	15.7	1.25	244	19.6	17.0	23.4	.110	1.88
64	8	62	9.7	20.7	1.25	360	25.9	19.0	22.1	.109	1.76
47	8	59	11.9	20.7	1.13	285	23.3	19.0	23.7	.111	1.69
2	8	87	9.5	20.7	1.50	492	31.0	19.0	22.5	.091	1.71
12	8	73	10.4	20.7	1.50	353	31.0	19.0	22.9	.101	1.39
20	8	79	12.3	20.7	1.50	395	31.0	19.0	24.5	.097	1.59
42	8	70	10.9	20.7	1.38	430	28.5	19.0	23.2	.103	1.90
41	8	71	11.1	20.7	1.25	339	25.9	19.0	23.4	.103	1.66
61	8	58	11.0	20.7	1.25	331	25.9	19.0	26.4	.112	1.99
43	8	74	11.6	27.8	1.38	457	38.2	21.2	23.8	.101	1.36
45	8	61	11.0	27.8	1.38	369	38.2	21.2	23.1	.110	1.16
63	8	62	8.7	27.8	1.38	497	38.2	21.2	21.3	.109	1.42
44	8	74	10.2	27.8	1.25	613	34.8	21.2	22.8	.101	1.91
46	8	60	11.4	27.8	1.13	497	31.3	21.2	23.3	.110	1.92
50	8	67	13.3	27.8	1.00	395	27.8	21.2	25.0	.106	1.78
57	8	73	11.0	27.8	1.00	465	27.8	21.2	23.3	.101	1.86
11a	5.8	71	11.6	20.7	1.38	222	28.5	16.7	23.8	.141	1.57
12a	5.8	71	11.7	20.7	1.25	235	25.9	16.7	23.8	.141	1.81
13a	5.8	71	11.0	15.7	1.25	171	19.6	15.1	23.3	.141	1.90
14a	5.8	71	11.1	15.7	1.50	149	23.6	15.1	23.4	.141	1.38
18a	5.8	88	4.6	27.8	1.25	457	34.8	18.7	18.6	.130	1.70
19a	5.8	89	5.3	27.8	1.13	436	31.3	18.7	19.2	.129	1.84
20a	5.8	89	5.5	20.7	1.50	320	31.0	16.7	19.4	.129	1.55
25a	5.8	94	5.0	15.7	1.75	236	27.5	15.1	19.1	.125	1.36
26a	5.8	92	4.6	20.7	1.13	297	23.3	16.7	18.7	.127	1.81
33a	5.8	91	5.7	27.8	1.38	395	38.2	18.7	19.5	.127	1.37
34a	5.8	89	5.4	15.7	1.38	222	21.6	15.1	19.3	.129	1.69
35a	5.8	89	5.0	15.7	1.63	242	25.5	15.1	19.0	.129	1.54
40a	5.8	84	7.1	27.8	1.00	347	27.8	18.7	20.6	.132	1.82
59a	5.8	81	7.2	20.7	1.38	277	28.5	16.7	20.6	.134	1.61
27a	7.8	92	5.1	15.7	1.75	391	27.5	16.7	19.1	.091	1.46
28a	7.8	91	5.4	20.7	1.38	395	28.5	18.7	19.3	.092	1.32
41a	7.8	84	6.4	15.7	1.38	250	21.6	16.7	20.0	.097	1.34
45a	7.8	85	6.0	15.7	1.63	244	25.5	16.7	20.2	.096	1.12
46a	7.8	85	7.0	15.7	1.50	256	23.6	16.7	20.5	.096	1.28

¹ According to equation (32) $C = \frac{R\gamma\bar{C}_p \ln \left[C_1 \left(\frac{t_f - t_o}{t_f - t_i} \right) \right]}{(f_c + f_r)\sigma L}$ where fuel density, γ , = 26.1 lbs./cu. ft.; t_f =

1,500° F.; t_i = 550° F.

² 1 + number of inches.

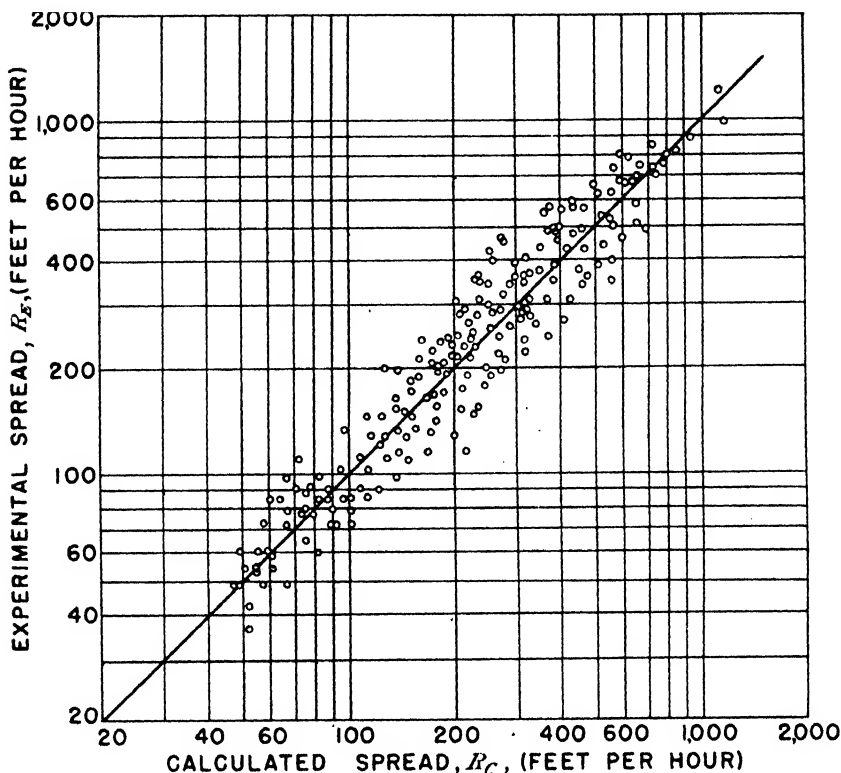


FIGURE 10.—Calculated versus experimental rates of forward spread for ponderosa pine-needle fires.

VARIABLES MEASURABLE IN FIELD PRACTICE

Equation (32) has been derived for the rate of spread in terms of the fundamental variables, which in practice are generally wind velocity, moisture content, time, and slope. Fuel-type or cover-type classifications also are used almost universally in recognizing the rate-of-spread differences attributable to fuel size and cover density, which the present study has revealed to be important and significant. Compactness, which incorporates fineness and crowdedness of the particles in the fuel bed, is used in this analysis to define cover density. Two other important measurable variables have been added, namely, fuel temperature and fuel-particle density, heretofore not generally recognized or applied in practice.

These measurable variables influence fire spread only as they produce changes in the fundamental variables. Some operate on as many as three or four fundamental variables. For example, the fuel temperature, t_o , affects film conductance for convection and conduction, f_c , heat-transfer factor, f_r , specific heat of moist fuel, \bar{C}_p , and in addition is itself a fundamental variable in the spread equation.

Figure 11 shows curves for rate of spread as a function of all the variables, except slope and time, as interpreted through application of the fundamental relationships expressed in equation (32).

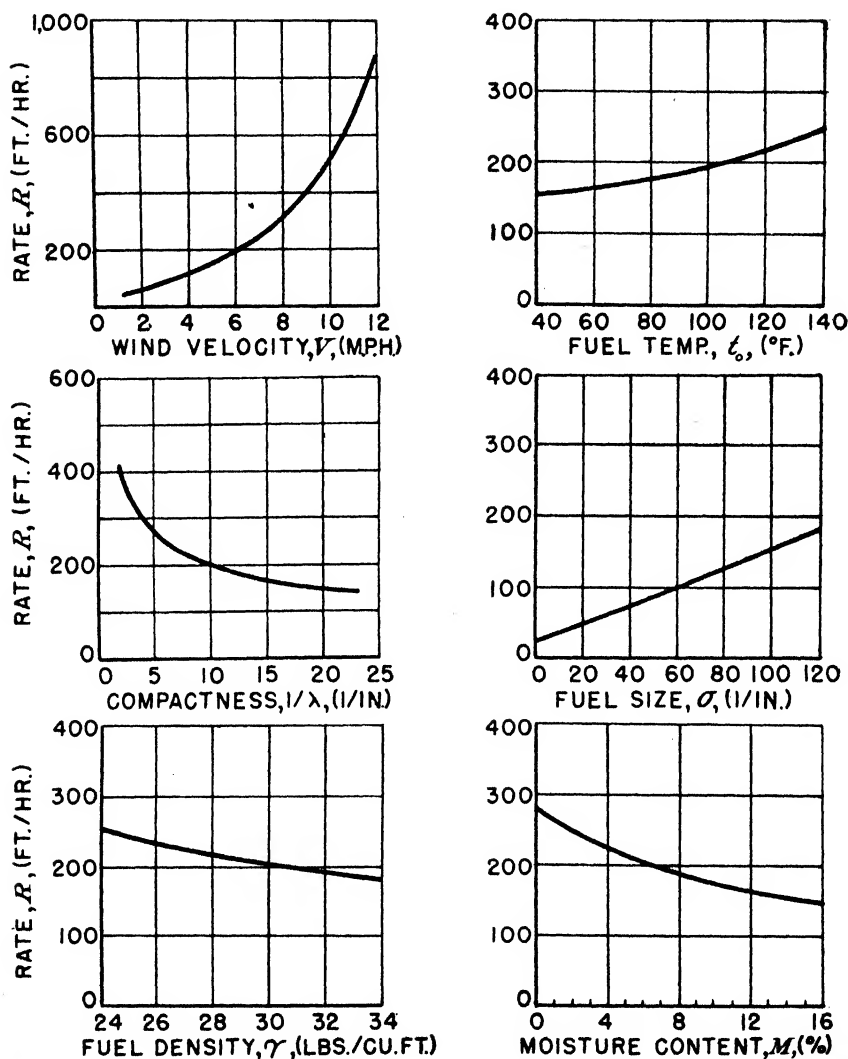


FIGURE 11.--Effect of measurable variables on rate of forward spread of fires. For each graph five variables are held constant while the sixth is varied over a practical range. When held constant, the variables have values as follows: $V=6.0$ m. p. h.; $1/\lambda=10$ inches⁻¹; $\gamma=31.6$ lbs./cu. ft.; $t_0=100^\circ$ F.; $\sigma=128$ inches⁻¹; $M=8$ percent.

INFLUENCE OF MEASURABLE VARIABLES

EFFECT OF WIND VELOCITY

Wind velocity influences fire spread by causing changes in the magnitude of film conductance, f_c , and temperature ratio coefficient, C_t . The film conductance varies approximately with the square root of the wind velocity (see equation 12). Since rate of spread is directly proportional to f_c , it will vary with the square root of the wind velocity. Most of the increase in fire spread that is due to the wind velocity, however, results from its effect on the temperature ratio coefficient, C_t . Increasing velocity decreases the flame angle, and this results in more effective preheating of the fuel; that is, fuel temperature, t_i , increases with wind velocity. From equation (29) it will be seen that higher temperature values t_i of the fuel particle about to ignite will give smaller values of C_t ; thus C_t is some inverse function of wind velocity. The way C_t varies with wind velocity is shown in figure 9. Since the rate of spread varies inversely as the logarithm of C_t , it will vary directly as the logarithm of the velocity. With wind measured at 1 foot above the fuel bed, the product of these separate functions indicates that rate of spread is approximately proportional to the first power of wind velocity for velocities less than 5 m. p. h. and to the 1.5 power of wind velocity for velocities from 5 to at least 12 m. p. h. The upper velocity limit (to which the 1.5 power holds) has not been definitely fixed. The first power for wind velocities less than 5.0 m. p. h. is confirmed by experimental results obtained by Curry and Fons (6) from test fires under field conditions.

EFFECT OF FUEL SIZE

In this analysis, the surface-volume ratio, σ , of the fuel particle has been used to express the fuel size. The presence of σ in the numerator in equation (32) does not necessarily imply that rate of spread is directly proportional to σ . In fuel beds of constant compactness, variations in σ will affect the distance L between fuel particles (equation 21). In addition, σ affects the film conductance (equation 12). The effect of σ on ignition temperature, t_i , has not been considered in the analysis, but significant variations of t_i with σ are expected (equation 10). By holding all other factors constant, the expression for rate of spread in terms of σ becomes:

$$R = (a\sigma + b\sigma^{0.47} + c)(1 + d\sigma)^{1/2}$$

where a , b , c , and d are the proper constants for the given values of wind velocity, initial fuel temperature, compactness, and moisture content. For fine fuels this expression very closely approximates a straight line having positive slope. Experimental results (7) show that the rate of spread for fine fuels is very nearly inversely proportional to the fuel size, i. e., it varies directly as the surface-volume ratio of the fuel particle.

EFFECT OF COMPACTNESS

For want of a more descriptive word, the variable incorporating fineness and crowdedness of the fuel particles is here termed compactness. The reciprocal of the ration of volume of voids to the surface

area of the fuel in the bed, denoted by λ , may be used as a direct measure of this element. By definition, the spacing of fuel particles, L , varies as the square root of λ . Since rate of spread is proportional to L , it too will vary as the square root of λ . For a fuel particle of given size it is necessary to increase the distance L between particles to obtain greater values of λ (equation 21); this would indicate that a unit increase at large values of λ would not increase the rate of spread as much as at the small values. There must be, of course, a limiting value of λ at which the fires would fail. Beyond a critical limiting point further separation of fuel particles decreases the volume of flame, and the hot gases produced in diminishing quantity are cooled and dissipated to such an extent that they are no longer effective in igniting the unburned fuel. Experimental results (7) have confirmed the square root of λ relationship.

EFFECT OF FUEL TEMPERATURE

Changes in the initial temperature of the fuel, t_o , affect the four fundamental variables, film conductance, heat-transfer coefficient, specific heat of the moist fuel, and temperature ratio $\frac{t_f - t_o}{t_f - t_i}$. The film conductance changes with the fuel temperature because the kinematic viscosity and thermal conductivity of the film are affected (equation 12). An increase of 50° F. in fuel temperature, t_o , will, however, decrease the film conductance only 0.5 percent and may be disregarded. The heat transfer coefficient, f_r , is also negligible for large changes in fuel temperature, because flame temperature is large in comparison with t_o (equation 14). The specific heat of the moist fuel, \bar{C}_p , increases with a rise in fuel temperature (equation 27). Since rate of spread is inversely proportional to C_p (equation 32), an increase in \bar{C}_p decreases the rate of spread. The important effect of fuel temperature on fire spread is that a change in temperature changes the ignition time, θ_i , as shown by equation (10), with

$C_i \left(\frac{t_f - t_o}{t_f - t_i} \right)$ replacing $\frac{t_f - t_i}{t_f - t_i}$. Changes in fuel temperature affect the magnitude of the ratio $\frac{t_f - t_o}{t_f - t_i}$, and a small change in this ratio produces a comparatively large change in $\ln \left[C_i \left(\frac{t_f - t_o}{t_f - t_i} \right) \right]$. The relative extent of the fuel temperature's effect on fire spread may be determined by calculating the magnitude of the factors $1/\ln \left[C_i \left(\frac{t_f - t_o}{t_f - t_i} \right) \right]$ and $\frac{f_c + f_r}{\gamma \bar{C}_p}$ in equation (32). For conditions indicated in figure 11, an increase in fuel temperature from 70° to 120° F. causes the factor $1/\ln \left[C_i \left(\frac{t_f - t_o}{t_f - t_i} \right) \right]$ and consequently rate of spread, to increase 35 percent; with the same change, the factor $\frac{f_c + f_r}{\gamma \bar{C}_p}$ causes a decrease of 5.5 percent. The total effect of the 50° F. increase in initial fuel temperature, under the conditions described, is thus a net increase of 29.5 per-

cent in fire spread. Since C_i decreases with wind velocity, a larger increase in spread results with rising wind velocity for a given temperature change; i. e., for the same conditions as above except that with wind velocity at 10 m. p. h. a rise in temperature from 70° to 120° F. produces a 94-percent increase in the rate of spread. The tendency of a fire to slow down soon after a shadow from a cloud is cast on the fuel ahead of the fire has frequently been observed. The fuel temperature in the rate-of-spread equation offers a possible explanation of this phenomenon, since fine fuels in complete shade soon assume air-temperature conditions, while in direct sunlight a much higher fuel temperature generally prevails.

EFFECT OF FUEL DENSITY

The density of the moist fuel, $\bar{\gamma}$, appears in the form of an inverse proportion as one of the fundamental variables in the expression for rate of spread. Since the density of a body is its mass per unit volume, more heat is required to bring a body of higher density than one of lower density to a given temperature. Consequently, the ignition time for fuel particles of a given volume is directly proportional to density (equation 10), and by the same token fire spread is inversely proportional to density.

EFFECT OF MOISTURE CONTENT

The moisture content, M , appears in the factors $1+M$ and $\frac{M}{1+M}$ in the equations for density and specific heat of the moist fuel, respectively. The factor $1+M$ in the two expressions cancels, leaving M in the product $\bar{\gamma}\bar{C}_p$, which is an expression for specific heat based on volume or volumetric specific heat. Since fire spread varies inversely with $\bar{\gamma}\bar{C}_p$ (equation 32), it will also vary with the fuel-moisture content. Moisture content can be expected to have the same effect on the rate of spread as density has, in that the greater the moisture the more heat is required to bring the fuel to the ignition point and the slower it will burn. Since rate of spread must become zero at some given moisture content for a particular fuel size, it is doubtful whether the effect of absorbed water on rate of spread is accomplished entirely through its effect on $\bar{\gamma}$ and \bar{C}_p for the complete range of moisture content. At high moisture contents it would seem probable that the excessive water vapor acts as a diluent for the inflammable fuel gases to the extent that rate of spread decreases more than $\bar{\gamma}$ and \bar{C}_p might indicate. If the moisture is sufficiently high, the water vapor may dilute the gases to the point where combustion is entirely prevented; this would determine the upper moisture content limit. For the range of moisture content studied (4 to 15 percent) equation (32) is valid; otherwise values of C_i computed for the experimental fires for any given wind velocities would have varied with the moisture. This, however, was not found; thus the effect of absorbed water on rate of spread is apparently accounted for by $\bar{\gamma}$ and \bar{C}_p for moisture contents up to 15 percent.

EFFECT OF SLOPE

Because hot gases or convection columns tend to rise vertically with respect to a horizontal surface, an increase in slope of terrain

decreases the angle between the fuel bed and hot gases. Slope, therefore, has the same effect as wind in bringing the flame closer to the fuel. As such, it may be considered an added component of wind velocity. As the flame angle decreases, the fuel starts to absorb heat, both by radiation and convection, farther ahead of the actual burning front than with a flame perpendicular to the fuel surface. This results in increasing t_1 , temperature of the fuel particle about to ignite, which in turn makes C_t smaller (equation 29). Since the rate of spread varies inversely as the logarithm of C_t , it will vary directly

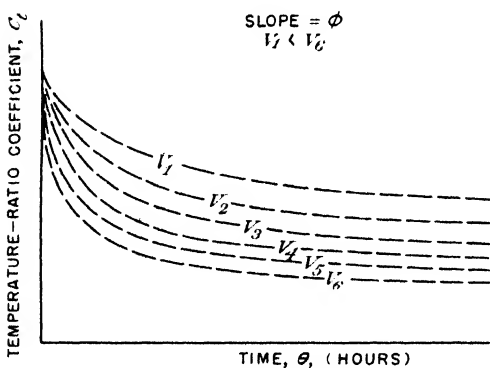
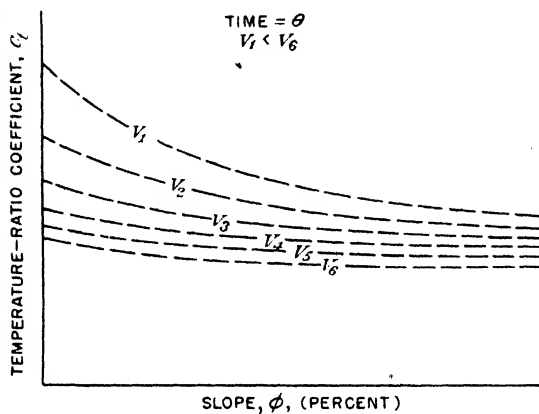


FIGURE 12.—Curves showing how the temperature-ratio coefficient may be expected to vary with slope and time for various wind velocities, V .

as the logarithm of time. This relationship was also indicated by earlier studies of field test fires (6). Model fires are of too short duration to permit studying the effect of time on C_t . Data from which to derive actual values of C_t at different time intervals must therefore be obtained from larger scale field experiments, which have not yet been undertaken. The way in which C_t is expected to vary with time for several wind velocities and given slope is illustrated in figure 12.

the logarithm of the slope. The effect of slope on rate of spread indicated by this relationship is shown in figure 12, where the lines drawn for different wind velocities are broken to indicate that their true positions have not yet been experimentally established.

EFFECT OF TIME

Immediately after the start of a fire the volume of hot gases at its head increases progressively. As the volume of the flame rises the rate of heat transfer by radiation to the fuel particles is increased; thus t_1 , increases with time until the volume of hot gases from the flame front has reached a more or less constant value. As in the case of slope, increasing values of t_1 result in decreasing values of C_t (equation 29); the rate of spread will therefore vary as

VALUES FOR GROUPS OF FUNDAMENTAL VARIABLES IN TERMS OF MEASURABLE VARIABLES

In table 2 are presented values of σ , γ , and E for different kinds of fuel. In tables 3 to 6, inclusive, are computed values of groups of fundamental variables that appear in equation (32), as functions of those measurable variables affecting the group.

TABLE 2.—Surface-volume ratio, perimeter, density, and shape factor of several fuels

Fuel type	Surface-volume ratio	Perimeter	Density	Shape factor
	σ	P	γ	E
Ponderosa pine:	¹ Inches ⁻¹	Inches	Pounds/cu. ft.	
Dead twigs	$\frac{4}{D}$	$2\pi D$	29.1	3.6
Square sticks	$\frac{4}{t}$	$34t$	26.7	4.0
Needles:				
Ponderosa pine	128	131	31.6	4.1
Sugar pine	165	108	33.5	4.2
Lodgepole pine	140	136	35.4	4.3
Fir	124	165	34.3	4.5

¹ 1 = number of inches.² Diameter of fuel.³ Thickness of fuel.TABLE 3.—Values¹ of f_s and f_r , (B. t. u./sq. ft. hr. °F.) as a function of wind velocity and surface-volume ratio of fuel

Surface-volume ratio (inches ⁻¹) ²	Wind velocity, in miles per hour indicated at 1 foot above fuel bed					
	2	4	6	8	10	12
10	9.7	11.8	13.5	14.8	16.1	17.2
20	11.5	14.5	16.8	18.9	20.7	22.2
40	14.3	18.5	21.8	24.5	26.9	29.1
70	17.6	22.6	26.8	30.7	33.8	36.8
100	20.3	26.3	31.3	35.2	39.0	42.3
130	22.7	29.4	34.9	39.2	43.6	47.2
160	25.1	32.2	38.4	43.5	47.9	51.8

¹ Values in table are within ± 0.5 percent for a range of fuel temperature from 50° to 140° F.² 1 = number of inches.TABLE 4.—Values¹ of $\frac{\sigma L}{E}$ as a function of surface-volume ratio of fuel and fuel bed compactness

Surface-volume ratio (inches ⁻¹) ²	Fuel bed compactness (inches ⁻¹) ²							
	0.2	0.5	1	2	4	6	10	20
10	7.14	4.58	3.32	2.45	1.87	1.63	1.41	1.22
20	10.05	6.40	4.58	3.32	2.45	2.08	1.73	1.41
40	14.18	9.00	6.40	4.58	3.32	2.77	2.24	1.73
70	18.73	11.87	8.43	6.00	4.30	3.56	2.83	2.12
100	22.38	14.18	10.05	7.14	5.10	4.21	3.32	2.45
130	25.51	16.16	11.45	8.12	5.79	4.77	3.74	2.74
160	28.30	17.92	12.69	9.00	6.40	5.26	4.12	3.00

¹ To obtain σL for equation (32), multiply $\frac{\sigma L}{E}$ by an appropriate shape factor, E , of the fuel.² 1 = number of inches.

TABLE 5.—Values¹ of $\frac{\gamma \bar{C}_P}{\gamma}$ as a function of moisture content and temperature of fuel

Fuel temperature (°F.)	Fuel moisture content (percent)							
	2	4	6	8	10	12	14	16
40.....	0.546	0.597	0.648	0.699	0.750	0.801	0.852	0.903
60.....	.556	.608	.660	.713	.765	.817	.870	.922
80.....	.566	.620	.674	.727	.781	.835	.888	.942
100.....	.577	.632	.687	.743	.798	.853	.908	.963
120.....	.588	.645	.702	.759	.816	.872	.929	.986
140.....	.600	.659	.717	.776	.835	.893	.952	1.010

¹ To obtain $\gamma \bar{C}_P$ for equation (32), multiply $\frac{\gamma \bar{C}_P}{\gamma}$ by the density of the fuel.

TABLE 6.—Values¹ of $\ln \left[C_i \left(\frac{t_f - t_o}{t_f - t_i} \right) \right]$ for surface fires as a function of wind velocity and fuel temperature, t_o , for time 1 minute from start of fire and zero slope

Fuel temperature (°F.)	Wind velocity (in miles per hour indicated) at 1 foot above fuel bed					
	2	4	6	8	10	12
40.....	0.2004	0.2078	0.1596	0.1249	0.0971	0.0760
60.....	.2769	.1939	.1458	.1106	.0834	.0620
80.....	.2631	.1798	.1319	.0971	.0695	.0478
100.....	.2484	.1664	.1178	.0825	.0554	.0344
120.....	.2343	.1519	.1035	.0686	.0411	.0198
140.....	.2199	.1371	.0889	.0535	.0266	.0070

¹ Flame temperature, t_f , and ignition temperature, t_i , are considered constant at 1500° and 550° F., respectively.

SUMMARY AND CONCLUSIONS

The analysis of rate of fire spread in light forest fuels reported in this paper is based on the theory that the spread of a fire can be expressed as successive ignitions of adjacent fuel particles and that its rate is therefore governed by the time required to raise successive fuel particles to ignition temperature. Equations have been derived for rate of fire spread in homogeneous and heterogeneous fuel beds, taking into account the physical characteristics of the fuel particles, the arrangement of the bed, and the pertinent attributes of the atmosphere. The spread equations are expressed in terms of the following fundamental variables: (1) Film conductance; (2) heat transfer factor for radiation; (3) ignition temperature; (4) fuel particle spacing; (5) surface volume ratio of fuel; (6) specific heat; (7) density of fuel; and (8) fuel temperature. It has been shown that the variables ordinarily recognized in field practice—wind velocity, fuel moisture, fuel density, fuel size, fuel bed compactness, slope, and time—influence fire spread only as they produce changes in the fundamental variables.

The results of field and laboratory experiments were used to check the theory and analysis, as well as to provide quantitative information for determining the magnitude of the effects of variables measurable in field practice on the rate of spread.

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A COMPARISON OF THE CHEMICAL COMPOSITION OF DIPLOID AND TETRAPLOID CORN¹

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INTRODUCTION

In crop production studies, increasing attention is being given to the evaluation of the crops produced in terms of constituents of nutritional significance, as well as in terms of total yield. Although extensive animal feeding trials are necessary to evaluate a crop completely, chemical data are useful indicators of the nutritional value of plants.

In 1940 Randolph and Hand (10)³ reported that doubling the number of chromosomes in pure yellow corn resulted in an increase of approximately 40 percent in the total carotenoid pigment content and in the biologically active provitamin A fraction of the grain. Further analyses on both the grain and stover of similar strains of corn indicate that doubling the number of chromosomes may bring about other changes in composition that appear also to be of nutritional significance.

MATERIALS AND METHODS

Analyses of the grain involved comparable diploid and tetraploid cultures in each of four stocks of corn (*Zea mays* L.), as follows:

A. A commercial inbred line of yellow dent corn, designated 4-8d, and an autotetraploid line derived from it through direct chromosome doubling induced by the heat-treatment technique of Randolph (9).

B. A commercial yellow dent line of tetraploid corn that was inbred for seven generations after it was produced by the heat treatment of a diploid hybrid combining inbred lines of Webber Dent, Illinois A, and Lucas Favorite, and diploid cultures originating from parthenogenetic diploid plants that arose spontaneously in the tetraploid line.

C. A first generation hybrid of the tetraploid lines of stocks A and B.

D. A white flint line of tetraploid corn that was inbred for four generations after it was produced by heat treatment of a diploid hybrid combining two similar lines of white flint corn, and diploid cultures originating from parthenogenetic diploid plants that arose spontaneously in this tetraploid line.

Analysis of the earfree stover involved A, B, and D utilized in the analyses of the grain and the following four additional stocks:

E. A commercial yellow dent tetraploid line, inbred two generations after being produced from a synthetic diploid hybrid involving inbred lines of Webber Dent,

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² Also professor of botany, New York State College of Agriculture, Cornell University.

³ Italic numbers in parentheses refer to Literature Cited, p. 130.

Illinois A, Lucas Favorite, and white flint, and a diploid culture that originated from a parthenogenetic diploid plant occurring in this tetraploid line.

F. Another tetraploid line of the same origin as E, inbred three generations, and a derived parthenogenetic diploid culture.

G. A third line of the same origin as E and F, inbred three generations, and a derived parthenogenetic diploid culture.

H. A tetraploid line of the same origin as B, inbred three generations and parthenogenetic diploid cultures derived from it.

The above stocks A to H were analyzed in 1940 (tables 1 to 3). In 1941 analyses of the stover in stocks A, B, and D were repeated in order to determine the extent of seasonal variation with respect to chemical composition, the same stocks being given different culture numbers in each of the 2 years as shown in table 3.

The samples of grain and stover were harvested at maturity, the grain being air-dried immediately after harvest to a moisture content of approximately 14 percent. The stover samples were taken from fully matured plants before they had been injured by frost or desiccation and while still green. After the stover samples had been air-dried, they were ground in a hammer mill. Suitable portions of this material and of the corn grain were prepared for analyses by grinding in a Wiley mill to pass through a 1-mm. screen.

All of the results given are expressed as percent on a dry matter basis. The dry matter was obtained by drying in a vacuum oven at 95° to 100° C. for 5 hours.

Total nitrogen was determined by the boric acid modification of the Kjeldahl method.

Ether extract, ash, and crude fiber were determined by the official methods of the Association of Official Agricultural Chemists (1).

The cellulose determinations were made by the Crampton-Maynard method (3) and the lignin determinations by a 72-percent sulfuric acid method, the detail of which have not yet been published. It consists essentially in extracting the air-dried sample with an alcohol-benzene mixture, followed by digestion with pepsin to render protein material soluble. After additional acid hydrolysis, the residue is treated with 72-percent sulfuric acid. Water is added to bring the sulfuric acid concentration to 3 percent and boiled. The residue after filtration and correction for its ash content is considered to be lignin.

RESULTS

The values for the nitrogen content of the corn grain are presented in table 1. This table and succeeding tables show that more than one culture of each stock ordinarily was analyzed. These cultures within each of the stocks originated from seed of selfed ears which were produced by sister plants in the preceding generation. For example, in stock B cultures 612 and 613 originated from the selfed seed of spontaneous parthenogenetic diploid plants, and cultures 614 to 618 from tetraploid sister plants of the same culture in the preceding generation.

In all comparisons, the nitrogen content was higher in the tetraploid stocks than in the comparable diploid stocks. The largest increase was in stock D, a white flint corn. The mean value with its standard error for all the diploid values was 1.799 ± 0.0887 , while the mean value for the tetraploids was 2.087 ± 0.0349 . The difference of 0.288 ± 0.0953 is statistically significant at odds of 100:1 and represents an average

increase of 15 percent. In this comparison, the values of stock C are omitted. These values are of interest since they show that this hybrid of the tetraploid stocks A and B was considerably lower in nitrogen content than either of the parent stocks.

The individual values recorded for stock C represent separate analyses and were included to indicate the adequacy of the sampling. The determinations were made on five replicate 20-gm. samples and show relatively close agreement among themselves. All the other samples were 50 gm. in weight with the exception of the tetraploids in stock A, which were 20 gm.

TABLE 1.—*Nitrogen content of corn grain, 1940*

Stock	Culture No.	Diploid nitrogen	Average	Culture No. ¹	Tetraploid nitrogen	Average
		<i>Percent</i>	<i>Percent</i>		<i>Percent</i>	<i>Percent</i>
A	091	2.139	2.139	562	2.309	2.22
				565A	2.173	
				565B	2.216	
B	612	2.016	1.999	614	2.042	2.15
	613	1.981		615	2.224	
				616	2.042	
				618	2.216	
C				575-1	1.714	1.75
				575-2	1.758	
				575-3	1.781	
				575-4	1.778	
				575-5	1.728	
D	601	1.522	1.651	603A	2.042	2.01
	602	1.523		603B	2.005	
	606	1.598		604A	2.082	
	607	1.646		604B	2.107	
	608	1.965		605	1.960	
				609	2.026	
				610	1.931	
				611	1.979	

¹ A and B in this column represent replicates of the same culture.

There was no significant difference between the tetraploid and diploid stocks with respect to ether extract, ash, or crude fiber, as indicated by the data presented in table 2.

TABLE 2.—*Composition of corn grain, 1940*

Stock	Culture No. ¹	Type	Ether extract	Ash	Nitrogen	Crude fiber
			<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
A	091	Diploid	3.96	2.00	2.139	2.66
	565A	Tetraploid	3.75	1.90	2.173	3.20
	565B	do	3.60	2.00	2.216	2.96
D	601	Diploid	5.37	1.77	1.522	1.68
	602	do	4.77	1.61	1.523	1.77
	603A	Tetraploid	5.33	1.66	2.042	1.74
	603B	do	5.40	1.59	2.003	1.79
	604A	do	5.58	1.60	2.082	1.89
	604B	do	5.56	1.70	2.107	2.15

¹ A and B in this column represent replicates of the same culture.

The values obtained for the nitrogen content of corn stover for both 1940 and 1941 are shown in table 3. The difference between the diploids and tetraploids with respect to nitrogen content was greater for the stover than it was for the grain. In all comparisons, with the

exception of stock G, the tetraploid stover was higher in nitrogen than the diploid stover. Thus, the mean value was 1.224 ± 0.0372 for the diploid and 1.464 ± 0.0319 for the tetraploid in 1940, and 1.452 ± 0.0882 for the diploid and 1.941 ± 0.0272 for the tetraploid in 1941. These values represent an increase in the nitrogen content of the tetraploid stover as compared with the diploid stover of 20 percent in 1940 and 34 percent in 1941. The seasonal difference with respect to the diploid and tetraploid cultures was greater for certain cultures than for others, but there was no apparent explanation for these differences.

TABLE 3.—*Nitrogen content of corn stover*

Stock	1940				Stock	1941			
	Culture No.	Diploid	Culture No.	Tetra-ploid		Culture No.	Diploid	Culture No.	Tetra-ploid
A	091	Percent 1.317	562 565	Percent 1.640 1.573	A	68A 68B	Percent 1.445 1.746	66A 66B 663A 663B 664A 664B 669A 669B 687A 687B 688A 688B	Percent 1.699 1.826 1.742 1.861 1.770 1.818 1.747 1.880 1.971 1.878 2.000 2.077
Average				1.607					
B	612 613	1.296 1.042	614 615 616 618	1.379 1.365 1.299 1.397					
Average		1.169		1.360					
D	601 602 606 607 608	1.117 1.211 1.283 1.173 1.045	603 604 605 609 610 611	1.627 1.584 1.646 1.238 1.230 1.328	Average		1.594		1.856
Average		1.166		1.442	B	409 413 459 464	1.458 1.773 1.366 1.595	407 408 410 411 412 414 660A 660B 457 458 460 461 462 463 465 466	1.792 1.661 1.885 1.970 1.962 2.224 1.813 2.227 1.734 1.821 1.960 2.064 1.939 2.054 1.947 2.104
E	416 420	1.298 1.347	417 418 419 422 423 424	1.619 1.432 1.422 1.622 1.184 1.509	Average		1.548		1.947
Average		1.318		1.465	D	448 454	1.102 1.130	445 446 447 451 452 453 456	2.176 2.038 1.920 2.269 2.002 1.970 2.150
F	431	1.173	432 433	1.864 1.770	Average		1.116		2.075
Average				1.817					
G	436	1.483	437 438 439 440	1.427 1.403 1.432 1.402					
Average				1.416					
H	425 426	1.355 1.000	427 428 429	1.382 1.314 1.453					
Average		1.178		1.383					
Mean of all values and standard errors.				1.224 \pm 0.0372	1.464 \pm 0.0379				
					1.452 \pm 0.0882				1.941 \pm 0.0272

Since the ears of the tetraploid plants were not as well filled as those of the diploid plants, a test was made to determine whether this fact had influenced the results. In the eight stover cultures designated by A and B (table 3, columns 7 and 9), samples were harvested from plants that produced no seed, the ear shoots having been protected

with glassine bags during the pollinating season, and from plants that did produce seed. The fertile plants are designated A; the sterile ones B. The ears in all cases were removed before analysis. The mean values with the standard error were 1.773 ± 0.0608 for the fertile and 1.914 ± 0.0559 for the sterile corn. This difference in nitrogen content is not statistically significant.

Certain samples of the corn stover that showed marked differences in the nitrogen content of the diploid and tetraploid cultures were subjected to additional analyses for ether extract, ash, crude fiber, lignin, and cellulose. The data are shown in table 4. The values for nitrogen content given in table 3 for these samples are included in table 4 for comparison but are expressed as crude protein. The factor 6.25 was used to convert nitrogen to crude protein. The results are expressed as crude protein so that the relationship between cellulose content and the nitrogenous constituents of the stover can be more readily seen. The only values other than those for crude protein content that show any appreciable differences are those for crude fiber and cellulose. Thus, in stocks A and D, the increased crude protein content is accompanied by a roughly corresponding decreased cellulose content. Where the increased crude protein is not balanced closely by a decrease in cellulose, a change in the amounts of other carbohydrates such as starch or the pentosans must occur. That the crude fiber determination is essentially a measure of the cellulose content of corn stover is indicated by the close parallelism between the crude fiber and cellulose values. Seasonal differences in cellulose and lignin content were not as pronounced as those for crude protein content.

TABLE 4.—*Composition of corn stover*

Stock	Culture No.	Type	Ether extract	Ash	Crude protein	Cellulose	Lignin	Crude fiber
			<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
A (1940)	091	Diploid	1.47	7.84	8.23	31.2	9.59	35.2
	562	Tetraploid	1.34	7.70	10.25	28.1	7.94	28.0
	565	do	1.39	7.43	9.83	28.9	8.86	29.8
	Average		1.37	7.57	10.04	28.5	8.40	29.9
D (1940)	601	Diploid	1.61	9.07	6.98	36.0	10.73	35.1
	602	do	1.48	8.63	7.57	35.9	10.50	35.0
	Average		1.55	8.85	7.28	36.0	10.62	35.1
	603	Tetraploid	1.69	8.79	10.17	29.0	9.66	25.4
	604	do	1.61	8.17	9.90	27.0	8.56	28.0
	605	do	1.71	9.76	10.29	31.3	10.10	30.1
	Average		1.67	8.91	10.12	29.1	9.44	27.8
F (1940)	431	Diploid		6.69	7.84	31.8	8.69	30.2
	432	Tetraploid		8.72	11.65	30.3	9.16	29.7
	433	do		7.54	11.07	30.6	8.19	28.4
	Average			8.13	11.36	30.5	8.68	29.1
D (1941)	448	Diploid			6.89	31.4	10.5	
	454	do			7.06	33.7	10.6	
	Average				6.98	32.6	10.6	
	445	Tetraploid			13.60	24.0	9.7	
	446	do			12.74	26.0	10.4	
	Average				13.17	25.0	10.1	

DISCUSSION

The data presented show clearly that tetraploid corn is higher in nitrogen and lower in cellulose than comparable diploid corn, and that both types have essentially the same amount of the fatlike constituents included in the ether extract and the inorganic constituents of the ash. But it is possible that these results do not accurately reflect the actual effect of chromosome doubling on the chemical composition of corn because of the influence of factors other than the difference in chromosome number. However, the experiments were carefully designed to minimize genetic and environmental differences that might affect the results. Various stocks were selected for study in order to sample different types of corn and also to include a sufficient number of comparisons to permit average differences between the diploid and the tetraploid to be obtained that would be more significant than differences based solely on one or a few comparisons.

Since stage of maturity can influence composition to a marked degree, the samples were harvested only from fully mature, green plants that had been grown in randomized plots in the same field. It is also possible that the tetraploid plants were more leafy than the diploids. If they were, this might have accentuated the observed differences, since the leaves probably contain more nitrogen per unit of dry weight than do the stems. Unfortunately, this ratio was not determined, but the diploid and tetraploid plants were not noticeably different in this respect.

The transformation from the diploid to the tetraploid state resulted in an appreciable change in the direction of higher nutritive values so far as can be judged by chemical analysis. Since the question of the significance of chemical analysis in evaluating feedstuffs has been adequately discussed elsewhere (3, 8), it is sufficient to point out here that at present a complete evaluation of nutritional quality is impossible without feeding trials with animals. In many instances, however, it is not feasible, for economic or other reasons, to subject experimentally grown plant material to animal tests. Chemical analyses in such cases are of value as indicators of nutritional quality. Much of the evidence available today indicates that a lower crude fiber and a higher nitrogen content both favorably influence nutritional quality.

From the genetic standpoint, the diploid and tetraploid cultures of stock A were strictly comparable in that the tetraploids originated by direct chromosome doubling of an inbred line of diploid corn that had been self-pollinated for many generations and was therefore essentially homozygous. The diploid and tetraploid cultures of the other stocks were not as strictly comparable as those of stock A. The tetraploid lines that produced the parthenogenetic diploids with which these other comparisons were made had not been inbred sufficiently to render homozygous all of the genes that may have influenced the chemical composition of the plants. But within most of these stocks more than a single diploid-tetraploid comparison was made, and it is believed that a sufficient number of stocks were involved in the experiments to furnish significant results, especially as the observed differences were consistent. With the exception of one of the seven stocks used in the protein analyses of the stover, the results exhibited trends which were all in the same direction for both nitrogen and

cellulose content. Analyses made in different seasons also exhibited similar trends.

The observed similarities and differences between the diploid and tetraploid corn can be explained on a genetic basis simply by assuming that certain genes affecting the chemical composition of the corn plant act in a cumulative manner and others do not. The genes which control nitrogen accumulation in the grain and in the stover apparently exhibited a cumulative effect in that the tetraploids, which have four doses of these genes in all parts of the plant other than the endosperm, and six doses of the endosperm genes, were consistently higher in nitrogen content than the related diploids, which have only two doses of these genes in the parts of the plant other than the endosperm and three doses in the endosperm tissue. The genes regulating the synthesis of the fatlike constituents of the ether extract and the accumulation of inorganic matter in the ash exhibited no dosage effect. Both the diploids and the tetraploids contained essentially similar amounts of these constituents.

The negative effect of chromosome doubling which was noted with respect to cellulose content of the cornstover was not unexpected. Percentage increases in certain constituents must be balanced by percentage decreases in other constituents. The decrease in the amount of cellulose corresponded roughly with the increase in crude protein ($N \times 6.25$) content of the tetraploid.

Randolph and Hand (10) accounted for an increase of 40 percent in the content of the carotenoid pigments in yellow corn meal on the assumption that the genes for yellow endosperm exerted a cumulative effect following chromosome doubling. The present investigation has demonstrated a similar but less pronounced increase in the nitrogen content of the corn grain and stover, which is interpreted in the same way.

Recently Barr and Newcomer (2) reported that tetraploid cabbage contains 36.48 percent more sugars, 23.86 percent more ascorbic acid, and 32.62 percent more colloidal nitrogen than diploid cabbage; but the diploid had about 14 percent more soluble nitrogen than the tetraploid. In experiments with autotetraploid *Nicotiana*, Noguti, Okuma, and Oka (7) and Noguti, Oka, and Otuka (6) reported that the tetraploids contained from 51 to 138 percent more nicotine than the corresponding diploids and that the content of nitrates, organic acids, sugars, total nitrogen, and ether extracts also was increased in the tetraploids.

In certain plants chromosome doubling apparently does not produce significant changes in chemical composition. For example, Kostoff and Axamitnaja (5) found that diploid and tetraploid petunias did not differ appreciably in the amounts of the more important chemical constituents. However, tetraploid tomatoes were reported by these workers to contain appreciably more water, nitrogen, proteins, and ash, but less carbohydrates than the related diploids. But the chemical composition of tetraploid tomatoes examined by Fabergé (4) was similar to that of the diploids.

If the chemical composition of plant tissues is regulated by the action of specific genes that may or may not act in a cumulative manner, it would be expected that the autotetraploids of certain species and of certain genotypes within species might differ from those of other species or genotypes with respect to chemical composition,

and others might not. Differences of this sort were noted in the present study and have been reported by other workers. Further investigations are needed to determine the general significance of chromosome doubling in relation to the nutritional value of cultivated plants.

SUMMARY AND CONCLUSION

In certain stocks of corn (*Zea mays* L.), doubling the number of chromosomes was accompanied by an increase in the amount of nitrogen present in the grain and in the stover, the average increase being 15 percent in the grain and from 20 to 34 percent in the stover.

The chemical composition of the grain in diploid and tetraploid maize was essentially the same with respect to ether extract, ash, and crude fiber.

The diploid and tetraploid corn stover contained essentially the same relative amounts of ash, ether extract, and lignin.

The higher crude protein ($N \times 6.25$) content of the tetraploid stover was accompanied by a correspondingly lower content of crude fiber and cellulose.

The changes in chemical composition that accompanied the transformation from diploidy to tetraploidy are assumed to be due to the cumulative action of certain genes, notably those concerned with protein metabolism, and the noncumulative action of other genes.

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THE RELATION OF TEST WEIGHT AND PROTEIN CONTENT TO THE MILLING AND BAKING QUALITY OF HARD RED SPRING WHEAT HYBRIDS¹

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INTRODUCTION

For several years data have accumulated at this station on the micro milling and baking quality of a large number of selections from hard red spring wheat hybrids. These hybrids originated in the wheat nursery at Fargo and have been submitted for technologic tests because of agronomic promise. The purpose of these tests is to obtain information on their suitability for commercial flour production. While many are found to be undesirable, as they show no improvement over varieties already grown commercially, a few are discovered each year which justify further study. If some of these wheats are proved to be equal or superior in quality to those already in production they may be included in plot trials at one or more localities in the State, and samples from these plots are then milled on the Allis-Chalmers experimental mill and the resultant flours baked by the 100-gm. method.

The present inquiry was undertaken to discover whether reliable preliminary information regarding milling and baking quality, as assessed by micro methods, could be secured from a knowledge of test weight per bushel and wheat protein content. The situation encountered in small-size nursery tests is quite different from the more common one of evaluating a number of varied samples consisting of only a few wheat varieties, and the relations between test weight and flour yield, and wheat protein content and loaf volume, existing in these samples might not be found in nursery material.

Comparisons between the standard and micro milling and baking methods reported in the literature increase confidence in micro methods. Geddes and Aitken (3),³ Geddes and Frisell (4), Harris and Sanderson (5), and Sibbitt, Scott, and Harris (8) have presented the merits of micro procedures and have compared the data with those obtained by the use of the Allis-Chalmers mill and 100-gm. baking method.

In the present paper the data secured from four successive wheat crops are discussed, and the information derived from micro milling and baking techniques in plant breeding work is pointed out.

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² The authors wish to acknowledge the valuable assistance of L. D. Sibbitt in performing the milling and baking tests, and of Muriel Elledge in analyzing the data.

³ Italic numbers in parentheses refer to Literature Cited, p. 135.

EXPERIMENTAL MATERIAL AND METHODS

The wheats studied were grown in 1940, 1941, 1942, and 1943 in the nursery rod rows at Fargo, N. Dak. They were all hard red spring wheats of diverse parentage. The individual wheat samples were hand-harvested and carefully protected from unfavorable weather while in the shock. They were threshed in a small, motor-driven thresher when dry and in suitable condition. There were 145 samples in 1940 and 1941, 214 in 1942, and 155 in 1943. The samples for technologic testing were each composited from the seed from several replications. Usually 250 gm. were received for milling and baking tests and nitrogen determinations.

The wheats were cleaned and tempered before milling. The milling and baking methods employed were those described by Sibbitt, Scott, and Harris (8). Test weight was ascertained by a micro method reported by Harris and Sibbitt (6) since the quantity of wheat was too small for the standard quart procedure, which requires approximately $1\frac{1}{8}$ quarts of grain. Total wheat protein was determined by the Kjeldahl procedure. Flour yield is expressed as percent of wheat at 13.5-percent moisture content. Wheat protein was calculated on a 13.5-percent moisture basis, while loaf volumes are from 25 gm. of flour containing 86.5 percent dry matter. The doughs were mixed in a Hobart C-10 mixer equipped with special dough hooks for mixing 50-gm. doughs. After mixing, the doughs were divided into equal portions by weight and fermented for 3 hours.

EXPERIMENTAL RESULTS

The individual determinations were too numerous for presentation, and are in themselves of no particular interest. The discussion of the results will therefore be limited to the means, the variability, and the relations between suitable pairs of the four variables: test weight per bushel, flour yield, wheat protein content, and loaf volume. In this discussion differences significant at the 5-percent point will be defined as significant, while those significant at the 1-percent point will be called very significant.

MEANS

The mean values for each variable studied for each year are shown in table 1. There are very significant differences among these annual values. The differences in test weight between 1940 and 1943 and between 1941 and 1942 are very significant, while those between 1940 and 1942 and between 1941 and 1943, are significant at the 5-percent point. These differences are principally caused by weather conditions, no doubt, although the experimental material varies in genetic composition from year to year. For flour yield all yearly averages differ very significantly, except those for 1942 and 1943, which are identical. There seems to be little association between the corresponding means for test weight and flour yield, despite a priori expectations. For wheat protein very significant differences exist between 1940 and 1941 and between 1941 and 1942, while the 1943 values are very significantly higher than any of the other three. The difference between the 1940 and 1942 means is just significant. There is possibly

some degree of association between the yearly averages for test weight and wheat protein since the 2 years of high test weight were those in which the protein was lowest, while low test weight crops apparently had high protein content. All loaf volume differences were very significant. Little relation between the wheat protein and loaf volume means are evident.

STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION

The standard deviations and coefficients of variation for each variable are shown in table 1 for each year. The significance of differences in variability between years was not tested, but there seems to be no extreme differences. Apparently variations in weather conditions and the genetic composition of the wheats did not greatly affect the variability of the results.

TABLE 1.—*Means, standard deviations, and coefficients of variation of test weight, flour yield, wheat protein, and loaf volume of wheats tested for each year.*

Crop year	Number of samples	Test weight			Flour yield			Wheat protein			Loaf volume		
		Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation
		<i>Lb. per bu.</i>	<i>Lb. per bu.</i>	<i>Lb. per bu.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Cc.</i>	<i>Cc.</i>	<i>Cc.</i>
1940	145	60.6	1.07	1.8	71.4	1.90	2.7	14.5	0.83	5.7	172	16.45	9.6
1941	145	59.8	1.13	1.9	70.2	2.12	3.0	15.3	.53	3.5	156	15.59	10.0
1942	214	60.3	1.36	2.3	72.4	1.66	2.3	14.7	1.07	7.3	167	17.94	10.7
1943	155	59.5	1.49	2.5	72.4	1.88	2.6	16.3	.62	3.8	187	17.35	9.3
Grand mean		60.1			71.7			15.2			170		

MAXIMUM AND MINIMUM VALUES

The maximum and minimum values of wheats tested, with corresponding ranges, are shown in table 2. It is realized that these values do not reflect the variability of the material as accurately as standard deviations since they are determined by very few variates and are subject to large errors of random sampling. They are presented merely to show the substantial differences in actual values found in a single crop year, and to illustrate the fact that absence of significant correlation between suitable pairs of variables was not caused by lack of variation in the material, which was all grown in the same location. Test weight varied 9.4 pounds per bushel in 1943, and flour yield varied 12.6 percent for the same crop. In 1942 wheat protein content differed 5.8 percent and loaf volume 120 cc.

The causes of these differences within years probably lie in variations in the genetic composition of the wheats, causing them to respond differently to weather conditions, since the soil properties should be strictly comparable for all samples.

CORRELATION COEFFICIENTS

The correlation coefficients between test weight and flour yield, and between wheat protein and loaf volume for each year are shown in table 3. Little comment on these is required, except to point out the

rather surprising lack of close association in most instances between the different pairs of variables. With the exception of wheat protein and loaf volumes for 1941 and 1943 these are too small to be of much practical significance. For instance, no worth while information regarding flour yield can be secured from a knowledge of test weight, and the only information of value from the test weight data is for grading and general descriptive purposes. The correlations between wheat protein and loaf volume are considerably better, but for the 1940 crop there is no relation of statistical significance, while for the 1942 crop the correlation, though significant, is of little practical value. For the remaining 2 years, 1941 and 1943, the correlations are relatively high but not high enough to permit the prediction of loaf volume from wheat protein with any reasonable degree of confidence. The relations between some of these associated data were examined for nonlinearity but no evidence of this was found.

TABLE 2.—*Maximum and minimum values of wheats tested with corresponding ranges of test weight, wheat protein, flour yield, and loaf volume for each year.*

Crop year	Test weight			Flour yield			Wheat protein			Loaf volume		
	Maximum	Minimum	Range	Maximum	Minimum	Range	Maximum	Minimum	Range	Maximum	Minimum	Range
	Lb. per bu.	Lb. per bu.	Lb. per bu.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Cc.	Cc.	Cc.
1940.....	62.7	57.2	5.5	75.2	64.9	10.3	17.8	12.4	5.4	220	140	80
1941.....	62.1	53.5	8.6	75.5	62.9	12.6	16.5	13.4	3.1	194	118	76
1942.....	63.6	55.8	7.8	77.0	67.0	10.0	16.8	11.0	5.8	235	115	120
1943.....	62.2	52.8	9.4	79.2	66.6	12.6	17.8	14.5	3.3	240	145	95

TABLE 3.—*Correlation coefficients between test weight and flour yield, and between wheat protein and loaf volume for each year*

Variables correlated		Crop year	Correlation coefficient r_{xy} ¹
X	Y		
Test weight (pounds per bushel).....	Flour yield (percent).....	1940	+0.108
		1941	+ .300
		1942	+ .230
		1943	+ .136
Wheat protein (percent).....	Loaf volume (cubic centimeter).....	1940	+ .076
		1941	+ .634
		1942	+ .205
		1943	+ .536

¹ The value of r_{xy} at the 5-percent point for the different years was as follows: 1940 and 1941, 0.163; 1942, 0.134; 1943, 0.158. Its value at the 1-percent point was: 1940 and 1941, 0.215; 1942, 0.177; 1943, 0.208.

DISCUSSION

The absence of high correlation between the two principal pairs of variables shows very clearly the fallacy of any but the most general and uncertain prediction of flour yield and loaf volume from test weight and wheat protein content, respectively. This is particularly true respecting test weight and flour yield. The correlation coefficients between wheat protein content and loaf volume for the 4 years were transformed and combined by the method suggested by Fisher

(2). The value obtained for the combined results was only $+0.370$. Since the square of this value is 0.137 , one could conclude that wheat protein content accounted for only approximately 14 percent of the loaf volume variation, indicating that about 86 percent is dependent upon other factors. The variation in flour yield independent of test weight would be greater because the seasonal correlations between these two variables are lower than for wheat protein content and loaf volume. Thus the plant breeder basing his estimates of the flour-yielding and baking capacity of his hybrids and selections upon the relatively easy and rapid determinations of test weight and protein content would be subject to substantial errors in his decisions regarding the milling and baking quality of his wheats, as determined by micro milling and baking methods, with the expenditure of needless time and expense in later eradicating inferior selections from the breeding program. In the interim there would be excellent prospects of some really promising varieties being discarded.

The results obtained from this investigation conducted with micro methods are in essential agreement with those secured by Hayes et al. (7) and Ausemus et al. (7) by the use of the Allis-Chalmers experimental mill and the 100-gm. baking method on wheats of diverse genetic origin grown at four Minnesota stations. However, Waldron and Mangels (9), also employing the Allis-Chalmers mill and the 100-gm. baking method, found significant relationships between flour protein content and loaf volume for nursery tests over a 4-year period ($r = +0.441$). Their values were not high enough, though, to be useful for predicting loaf volume from flour protein content.

SUMMARY AND CONCLUSIONS

Very significant differences between crop years were found in test weight, wheat protein content, flour yield, and flour loaf volume for 659 samples of hard red spring wheat produced in the Fargo nursery over a 4-year period. These differences were no doubt caused principally by yearly variation in weather conditions, with variations in the genetic composition of the wheats being less important.

Correlation coefficients for each of the four crop years between test weight and flour yield were so low as to be of no practical significance, while the coefficients between wheat protein content and loaf volume, though generally higher, were of little practical value for predicting loaf volume from wheat protein content. The relationships between the different pairs of variables were essentially linear in character.

The results secured in these investigations show the danger of relying solely upon test weight and wheat protein content data to predict accurately milling and baking quality in nursery rod-row samples of hard red spring wheat.

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INFLUENCE OF CARBOHYDRATE LEVELS AND ROOT-SURFACE MICROFLORAS ON PHYMATOTRICHUM ROOT ROT IN COTTON AND MAIZE PLANTS¹

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INTRODUCTION

During its fruiting period cotton (*Gossypium* spp.) is highly susceptible to attack by the root rot fungus *Phymatotrichum omnivorum* (Shear) Dugg. The fact that *P. omnivorum* attacks some 2,000 species of plants (23, 26)³ indicates that the parasitic activity of this fungus is not highly specialized. Under sterile conditions it makes a vigorous vegetative growth on diverse synthetic media (3, 12, 24, 27) and forms sclerotia when grown on synthetic media as well as on various plant materials (7, 17). The organism has been observed to die out in decaying organic material, but food exhaustion is not primarily responsible for this result (20); with the removal of competitive effects of other microbes by sterilization, the fungus grows well after reinoculation, regardless of the amount of decomposition that the material has previously undergone.

The actual penetration of *Phymatotrichum omnivorum* into the cells of a host plant is preceded by enzymatic and other chemical action that kills the protoplasts in advance of cell-wall destruction (27, p. 59; 32, 33). The foregoing findings, taken in conjunction with the fact that the organism does not become systemic in its host, point to the conclusion that it not only does not require but that it never utilizes living materials as such. In other words, this parasite appears to function as a saprophyte abundantly equipped with extracellular enzymes.

The conclusion that death of the cell precedes penetration points to an additional probability, namely that the action of *Phymatotrichum omnivorum* on and in roots releases nutrients favorable to the growth of other micro-organisms that advance with it. The existence of *P. omnivorum* within attacked tissues would thus be highly competitive, and its growth and survival would depend largely upon the relative suitability of the tissue substrate for this fungus and for competitive or antibiotic saprophytes.

The investigation reported here deals with (1) the relation between carbohydrate levels and susceptibility of cotton to phymatotrichum

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³ Italic numbers in parentheses refer to Literature Cited, p. 160.

root rot, (2) the effects of carbohydrates in the roots of the cotton plant on the root-surface microflora, and (3), by means of sterile cultures, the protection afforded by the root microflora against the invasion and growth of the fungus on the roots of young cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.) plants.

REVIEW OF LITERATURE

It is well known that some of the obligate parasites grow most vigorously in normal, well-nourished, host plants. Literature showing poor growth of rust and mildew in leaves low in carbohydrates has been reviewed by Trelease and Trelease (31). Somewhat less is known about the relation of the root-infecting fungi to the composition of the host plant. Leach (18) has reported that, while *Armillaria mellea* can develop only on roots high in carbohydrates, *Rhizoctonia bataticola* and *Botryodiplodia theobromae* are capable of developing in roots of low carbohydrate content. Infections by *A. mellea* were reduced in newly planted land by ring-barking the trunks of forest trees prior to clearing for tea gardens. According to Garrett (13, p. 44), Gadd has shown that failure of tea plants to recover after too frequent plucking and pruning appeared invariably to be associated with the absence of starch reserves in the roots and that this condition actually preceded infection by *Botryodiplodia*. Roots infected by *Poria*, *Rosellinia*, and *Ustilina* showed normal starch content.

Several investigators have given consideration to relations between susceptibility to phymatotrichum root rot and carbohydrate levels in roots and in nutrient media. Blank (2) ascribed the resistance of young cotton plants to their low carbohydrate content. The addition of carbohydrates to agar and soil substrates supporting germinating cotton seeds has been said by Watkins (32) and Blank (2) to increase infection. Watkins and Watkins (33, 34) reported ready infection of seedlings of cotton, corn, and retama when germinated on agar rich in carbohydrates. Reduction in the viability of *Phymatotrichum omnivorum* on cotton roots following girdling has been considered by Ezekiel (11) in connection with an associated reduction in the carbohydrate concentration of the roots. Ergle (9), Ergle et al. (10), and Talley⁴ have found the carbohydrate concentrations in cotton plants to vary with age.

The general subject of disease protection by root microflora has been considered by various investigators. Lochhead (19) believed it possible that resistance to certain root diseases may be connected with the selective action of root excretions upon the saprophytic microflora, thus favoring types that may be more antagonistic to pathogenic micro-organisms in some cases and in others less. The presence of hydrocyanic acid in solutions in which resistant varieties of flax had been grown led Timonin (30) to suggest that root excretions of cyanogenetic glucosides or of hydrocyanic acid from resistant varieties either affect pathogenic fungi directly or else stimulate other micro-organisms, which in turn may influence or control the activity of pathogenic fungi.

That the number of micro-organisms associated with plant roots changes with the aging of the plants has been demonstrated by

⁴TALLEY, P. J. [Unpublished data.]

Starkey (22) and Mitchell, Hooton, and Clark (20). The last-named investigators noted also that in mechanically injured cotton plants the number of micro-organisms associated with root surfaces increased greatly during the late summer or early fall. In a continuation of this work Clark (5) found a change in types of saprophytic fungi as well as increased difficulty of recovery of *Phymatotrichum omnivorum* after injury. In a paper on the take-all disease of cereals Garrett (14) pointed out that the interest in microbiological antagonism has not led to new control measures of practical value. However, the value of organic manures in stimulating the soil microflora, in helping to alter the microbial equilibrium, and in hastening the elimination of undesirable fungi from soil, presumably through microbial antagonisms or competition, has been emphasized (6, 16); and changes in root-surface microfloras with changes in the soil microflora in response to the addition of soil amendments have been discussed (6). An appreciation of the significance of interactions between organisms may frequently be essential to the interpretation of experimental results and thereby serve to orient root-disease investigations.

MATERIAL AND METHODS

INOCULATIONS

For a study of the differences or changes in the susceptibility of plants to injury by *Phymatotrichum omnivorum*, an ideal inoculum is one that introduces the organism into each substrate uniformly as to quantity, activity, virulence, and amount of reserve food material. In the past, sclerotia, which are variable in size and activity, and pieces of infected root or other plant material, which are variable in nutrient reserve and in age of fungus, have been used as inoculum in a variety of ways. These past procedures did not seem to meet the requirements of the present investigation, and a new method was accordingly devised. By the procedure here described, a like number of milo (*Sorghum vulgare* Pers.) seeds infected with the fungus were introduced into the soil substrates at measured depths. The underlying purpose of this procedure was to make the number of days from inoculation to permanent wilting a reliable measure of the rate of upward growth of the fungus on the roots of the plant as well as of the ability of the fungus to invade and destroy.

The inoculum to be used was prepared by placing a layer of large milo seeds (previously autoclaved for 15 minutes with an equal weight of water) on well-established nutrient-agar plates of *Phymatotrichum omnivorum*. After an incubation period of 6 to 10 days at 28° C., the fungus was always well established in the seed, and there still remained within the seed a reserve of food for the subsequent development of mycelium.

In inoculating cotton plants growing in potted soils, three infected seeds were separated from the plate and dropped to the bottom of each of two holes made in the soil with a smooth, blunt rod. These holes were 8 to 16 cm. in depth, according to the age of the plants, and were placed 3 cm. on either side of the taproot. They were filled in with loose soil, and water was added to the soil surface. The sterile cultures were inoculated in a transfer room by pushing, with a sterile glass rod, three seeds through the glass watering tubes into the substrate. Importance was attached to uniform procedures

within each experiment, but the several experiments cannot be closely compared because of temperature and other differences affecting fungus activity.

CHEMICAL ANALYSES

The plant samples were preserved and the sugars extracted with hot 80-percent alcohol. Lead acetate and sodium oxalate were used in clarifying and deleading the solutions. The analyses for total sugars were made on solutions that had been inverted with hydrochloric acid for 24 hours at room temperature. Starch was determined by treating for 1 hour, boiling, and re-treating the residues, after sugar extraction, with diastase⁵ at 55° C. until the material gave no color with iodine solution. The residues from the starch hydrolysis were then refluxed with 2-percent hydrochloric acid for 2.5 hours, and the sugars obtained were reported as hemicelluloses. When starch was not determined, the residues from the alcoholic extraction were hydrolyzed with 2-percent hydrochloric acid, and the results were expressed as polysaccharides, the latter being equivalent to the sum of starch and hemicelluloses as determined separately in the other procedure.

The sugar analyses on plants grown in the greenhouses were conducted according to the method of Wildman and Hansen (35), except that potassium permanganate oxidizing solution and orthophenanthroline indicator were used in the titration. For the field samples, the cuprous titration method of Schaffer and Hartmann was employed.

STERILE CULTURES

Those who have attempted to grow plants on sterile substrates are familiar with the many difficulties involved. In the present undertaking it was necessary to select a substrate other than coarse sand because cotton plants growing in nonsterile sand succumb slowly and irregularly to root rot after inoculation.

The wilting of plants provides a good criterion of root destruction, and it was therefore regarded as desirable, if not essential, that the tops of the plants should be freely exposed to the greenhouse atmosphere; this required provision for frequent additions of sterile water. The sterile-culture procedure finally developed represents a composite of points of technique derived from the literature and new adaptations regarded as advantageous to this particular study.

A sand-bentonite mixture was extensively employed as the substrate for the plants, but in the early phases of the study soil was also used as a substrate. A good growth of plants was obtained after repeatedly washing soils in the autoclave, drying, and then twice dry-autoclaving the culture jars after they were filled, but the percentage of sterile cultures by this procedure was always low. The sand-bentonite mixture consisted of 80 percent of 20- to 40-mesh quartz sand, 15 percent of pulverized quartz sand, and 5 percent of bentonite. To reduce alkalinity, the ground quartz sand was treated with acid and washed. Iron was supplied by mixing 0.3 percent of magnetite with the substrate material, and Hoagland's nutrient solution was added to the substrate before it was autoclaved. The hydrogen-ion concentration of the substrate was usually within the pH range 7.0 to 7.6.

Two-quart mason jars were used for vessels in all sterile-culture tests (see fig. 5). Each of the jars was fitted with an 8-mm. glass

⁵ Undiluted diastase with low sugar content furnished by courtesy of Parke, Davis & Co

tube through which the plants were watered, inoculated, and sampled for sterility. The lower end of the tube extended about 6 cm. beneath the surface of the substrate through a glass sleeve (2 cm. in diameter by 6 cm. long) filled with coarse sand that served both for anchorage and for the distribution of water. The central portion of the watering tube was wrapped with cotton and tied inside the neck of the culture jar. The upper end of the tube, which extended well above the culture jar, was covered with a glass cap on a cotton shoulder. This cap was removed only in a transfer room. Cotton shoulders were tied around the outside of the neck of the culture jars and fitted with 600-ml. beakers with kraft-paper aprons. These beakers remained in place during autoclaving and after planting until the plant stems extended well above the tops of the jars.

Maize seeds and acid-delinted Stoneville 2-B cotton seeds were surface-sterilized in 6-gm. lots in 50-ml. Erlenmeyer flasks by covering them with 0.2-percent mercuric chloride in 50-percent alcohol and shaking vigorously for 2 minutes. This solution was then decanted off, and the seeds were washed three times with sterile water. After being washed, the seeds were covered a fourth time with sterile water and allowed to swell for 18 hours. The water was then decanted, and the seeds were transferred to potato-dextrose agar in Petri dishes for 40 hours. Uniformly germinated seeds from clean plates were selected for planting, and those with either shorter or longer hypocotyls were left on the plates for a further check on sterility. With this method, contaminations in the agar plates were rare and none of the germinated seeds from clean plates developed organisms when transferred to beef-extract peptone solution. The germinated sterile seeds were placed in depressions in the surface of the cultures and covered with the substrate. The control cultures were always treated and planted in the same manner as the sterile cultures, except that a small quantity of fresh soil was mixed in to reinoculate them after autoclaving and the beakers and cotton and paper covers were omitted to avoid seedling infections (damping-off and molds).

When the tops of the plants were well above the shoulders of the jars, the beakers were removed in a transfer room and sterile cotton pads were pressed firmly into place in the necks of the jars around the plant stems. The cotton shoulders that supported the beakers were removed at this time, and two sheets of light paper were then tied over the cotton pads. A second thin layer of cotton was placed above the paper, and over this a second paper covering. All these coverings were carefully fitted around the plant stems. The cultures were then weighed, to provide a basis for subsequent watering, and placed in the greenhouse in constant-temperature baths set at 28° C.

Much difficulty was encountered at first in maintaining sterile substrates with the tops of the plants freely exposed, but in the later experiments half or more of the cultures usually remained sterile for sufficiently long periods. It was customary to take samples of the substrate for plating at the time of planting and inoculation and subsequently at the times of watering. The latter samples were collected through the watering tubes by means of sterile glass tubes fitted with glass plungers. The increase in number of contaminated cultures with time may have represented contaminants introduced either by watering and sampling operations or by the infiltration of organisms along the plant stems.

CARBOHYDRATE LEVELS AND ROOT ROT

GREENHOUSE EXPERIMENTS

COTTON PLANTS OF FRUITING AGE

In two explanatory greenhouse experiments, plants of Rogers Acala cotton (*Gossypium hirsutum*) were grown in stone jars filled with 3 parts of Houston Black clay and 1 part each of Lufkin fine



FIGURE 1.—Cotton plants of the first greenhouse experiment 13 days after inoculation: A, Fruited plants with half of each leaf cut away; B, fruited control plants; C, plants with all branches and bolls removed.

sandy loam, river sand, and well-decomposed manure. The pots were weighed several times each week as a watering guide to avoid any important differences in the moisture content of the soil.

In experiment 1 there were three treatments (fig. 1), each with one plant in each of seven 2-gallon jars, as follows: A, Fruited plants

with half of each leaf cut away; B, fruited control plants; C, plants with all branches and bolls removed. The results of experiment 1 are shown in the following tabulation:

Treatment: ¹	Average period (days) from inoculation to wilting ²
A. Half leaf	12.7
B. Control	13.4
C. Branches and bolls removed	19.4

¹ Planted Sept. 20; treatments started Dec. 17; inoculated Jan. 18 at depth of 15 cm.

² Difference required for significance at 1-percent level, 5.8 days.

In experiment 2 there were two treatments, each with one plant in each of eighteen 1-gallon jars. Five days after inoculation, three jars from each treatment were selected at random for analyses of the root bark. The treatments were as follows: A, Fruited plants with two-thirds of each leaf cut away (starting 10 days before inoculation); B, plants with all branches and bolls removed (starting 10 days before inoculation) and given supplementary illumination of about 400 foot-candles (measured at the elevation of the upper leaves) for 4 hours in the early morning and 4 hours in the late afternoon. The results of experiment 2 are shown in table 1.

TABLE 1.—*Effect of treatments on carbohydrate concentrations in the root bark and on resistance of cotton plants to phymatotrichum root rot; experiment 2*

[Planted Oct. 3; treatments started Jan. 31; inoculated Feb. 11 at depth of 12 cm.]

Treatment	Root-bark carbohydrates ¹			Average time from inoculation to wilting
	Total sugars	Polysaccharides	Total	
A. Plants with one-third of leaf area	Percent 1.48	Percent 3.75	Percent 5.23	Days 17.1
B. Plants with main-stalk leaves only	2.41	11.31	13.72	(²)

¹ These samples were taken 5 days after inoculation and at that time there were few lesions on the roots. Percentages expressed on fresh-weight basis.

² All plants were alive on the 50th day.

The results of experiments 1 and 2 were alike in indicating that high-carbohydrate cotton plants are more resistant to root rot than low-carbohydrate plants. The differences in the carbohydrate levels in the root bark were probably more marked in experiment 2 than in experiment 1 because of greater increases in carbohydrates after sampling due to extra illumination of the B plants in experiment 2 and to the smaller quantity of nitrogen supplied after inoculation.

All the high-carbohydrate plants of experiment 2 were alive 50 days after inoculation, but when the roots were removed from the soil it was found that all had been actively infected (fig. 2) at some earlier stage of the test. The progress of the disease under treatment B had evidently been slow from the start and eventually was checked, as indicated by the marked overgrowth of the bark above the rotted portions of the roots. This overgrowth was not unlike that which appears above stem girdles, and it is reasonable to believe that the final checking of the disease was associated with carbohydrate concentrations in excess of the 13.72 percent found 5 days after inoculation; a further accumulation of carbohydrates logically would follow the destruction of the lower root system.

A third experiment was projected for the twofold purpose (1) of providing four carbohydrate levels and thereby aiding a differentiation between any effects on root rot resistance that might be associated

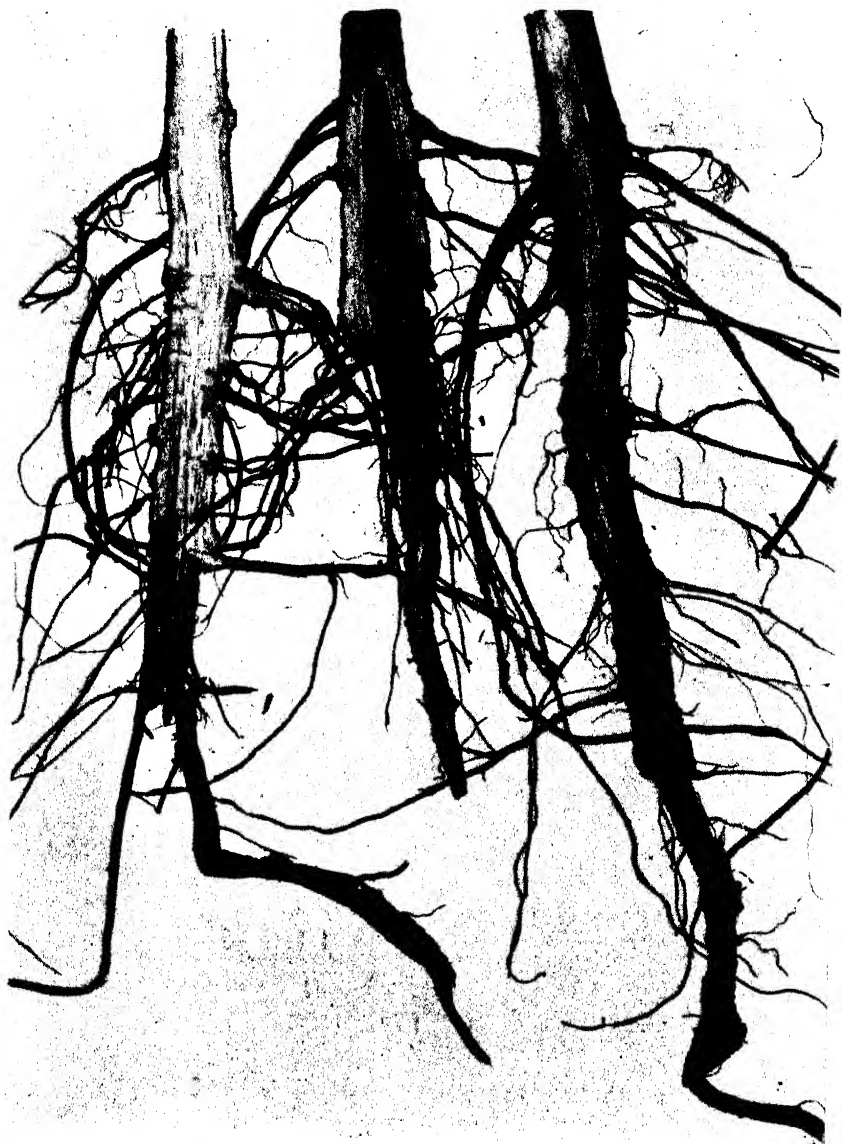


FIGURE 2.—Roots from the high-carbohydrate cotton plants of the second greenhouse experiment (table 1), showing checking of the disease and overgrowth of root bark.

with the mutilations or nitrogen accumulation, as distinct from the carbohydrate effect, and (2) of providing material for microbial assays and thereby the determination of possible relations between carbohydrate levels and equilibria of the root-surface saprophytes.

The four treatments were as follows: A, Fruited plants, normal nitrogen; B, fruited plants, low nitrogen; C, mutilated plants (all branches, bolls, and terminal buds removed), normal nitrogen; D, mutilated plants (all branches, bolls, and terminal buds removed), low nitrogen.

The seeds (Stoneville 2-B) were planted in 3-gallon jars containing 1 part Houston Black clay, 2 of Lufkin fine sandy loam, and 1 of sand. An equal amount of complete nutrient solution was added to all jars shortly after the seeds were planted, and during their early growth the plants were green and vigorous. By the time the first bolls were formed, however, growth was being checked by nitrogen deficiency. When there were from 2 to 4 bolls per plant, the pots

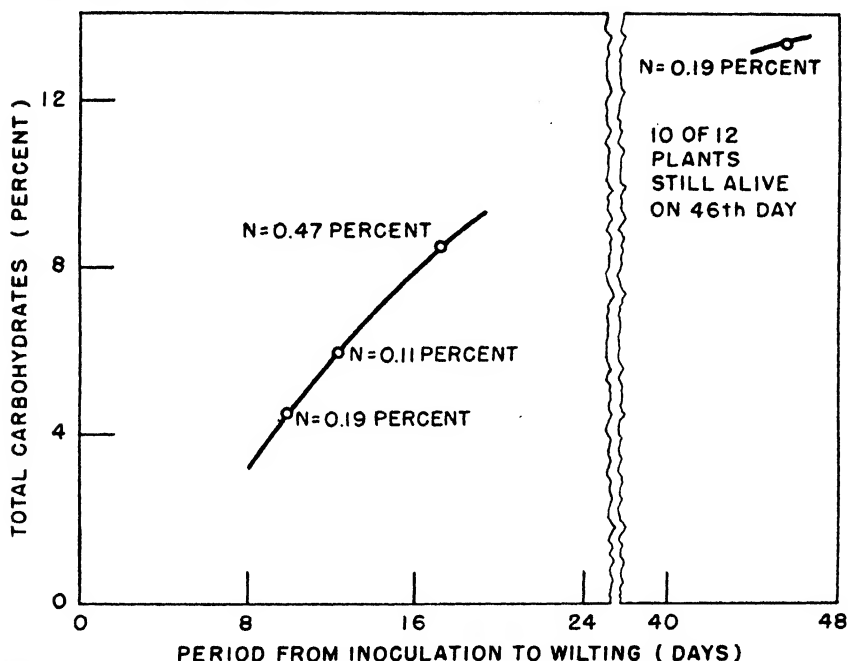


FIGURE 3.—Survival of cotton plants with successively higher carbohydrate levels in their root bark. Nitrogen concentrations in the root bark are shown numerically at each point.

were randomized into 4 groups of 17 each (except group D, 18 pots) and the differential treatments were started by adding a complete nutrient solution to groups A and C (normal-nitrogen plants) and only potassium and phosphate to groups B and D (low-nitrogen plants). All branches, bolls, and terminal buds were removed at this time from groups C and D (mutilated plants).

Sixteen days after the differential treatments were started and just prior to inoculation, six representative plants were selected from each treatment to provide duplicate samples of three plants each for the chemical analyses and microbial assays. The roots of these plants were collected 5 days after the plants to be tested for susceptibility had been inoculated. One-half of each root-bark sample, taken longitudinally, was used for chemical analyses (table 2 and fig. 3) and the other half for microbial assays (see table 7). The root samples were collected at 7 a. m.

TABLE 2.—Effect of nitrogen supply and mutilations on carbohydrate and nitrogen concentrations in the root bark and on resistance of cotton plants to *Phymatotrichum* root rot, experiment 3

[Planted Oct. 3; differential treatments started Jan. 6; inoculated Jan. 22; sampled Jan. 27]

Treatment	Root-bark composition ¹				Average period from inoculation to wilting
	Total sugars	Polysaccharides	Total carbohydrates	Total nitrogen	
	Percent	Percent	Percent	Percent	Days
A. Fruited; normal N.....	1.24	3.21	4.45	0.19	10.0
B. Fruited; low N.....	1.96	3.88	5.84	.11	12.4
C. Mutilated; normal N.....	1.77	6.57	8.34	.47	17.4
D. Mutilated; low N.....	2.23	10.82	13.05	.19	(²)

¹ Percentages expressed on fresh-weight basis.² 2 of the 12 plants died on the 21st and 22d day, respectively. The others remained alive.

The relation found in experiment 3 between carbohydrate accumulation in the root bark and the rapidity of kill by the fungus was notably direct, the successively higher carbohydrate concentrations being paralleled by corresponding delays in plant death. Of the 12 high-carbohydrate plants (treatment D) 10 remained alive.

The 10 high-carbohydrate plants that remained alive were subjected to some further experimentation. Forty-six days after inoculation, nitrogen was added to 5 of these pots and the plants were allowed to produce new branches. Of the 5 plants 4 died of root rot within the next month (2 died in 8 days and 2 in 26 days) and 1 remained alive. A root section of the surviving plant is shown in figure 4. Nitrogen was added to the remaining 5 pots 122 days after inoculation, and from that date on the plants were allowed to develop new branches. None of these plants died, and 3 that were not pulled out for root examinations set and matured a crop of cotton on their new branches. This fact shows that the root rot fungus, given time, will eventually disappear from diseased cotton roots if the carbohydrate concentrations are maintained at a sufficiently high level.

The results of the experiment (table 2 and fig. 3) indicate that differences in nitrogen supply and in growth habit affected the susceptibility of these cotton plants to root rot only as they affected the carbohydrate levels in the roots. The most susceptible plants (group A) and the most resistant ones (group D) had equal total nitrogen concentrations, whereas the plants of intermediate susceptibility (groups B and C) had the lowest and highest nitrogen levels, 0.11 and 0.47 percent, respectively. Moreover, there was no apparent break in the continuity of the carbohydrate root rot curve (fig. 3) in passing from nonmutilated to mutilated plants. In other words, a smooth curve was obtained through the first three treatments irrespective of the means (nitrogen or mutilation) adopted for varying the carbohydrate levels.

Under other conditions Streets (23), by using heavy applications of nitrogen, was able to invigorate trees infected with *Phymatotrichum omnivorum*, and Adams et al. (1) found reductions in root rot on some, but not all, soils when nitrogen was applied with a drill a few inches from the cotton seed at time of planting. The mechanism of the protective action of nitrogen sometimes observed in the field is not known.

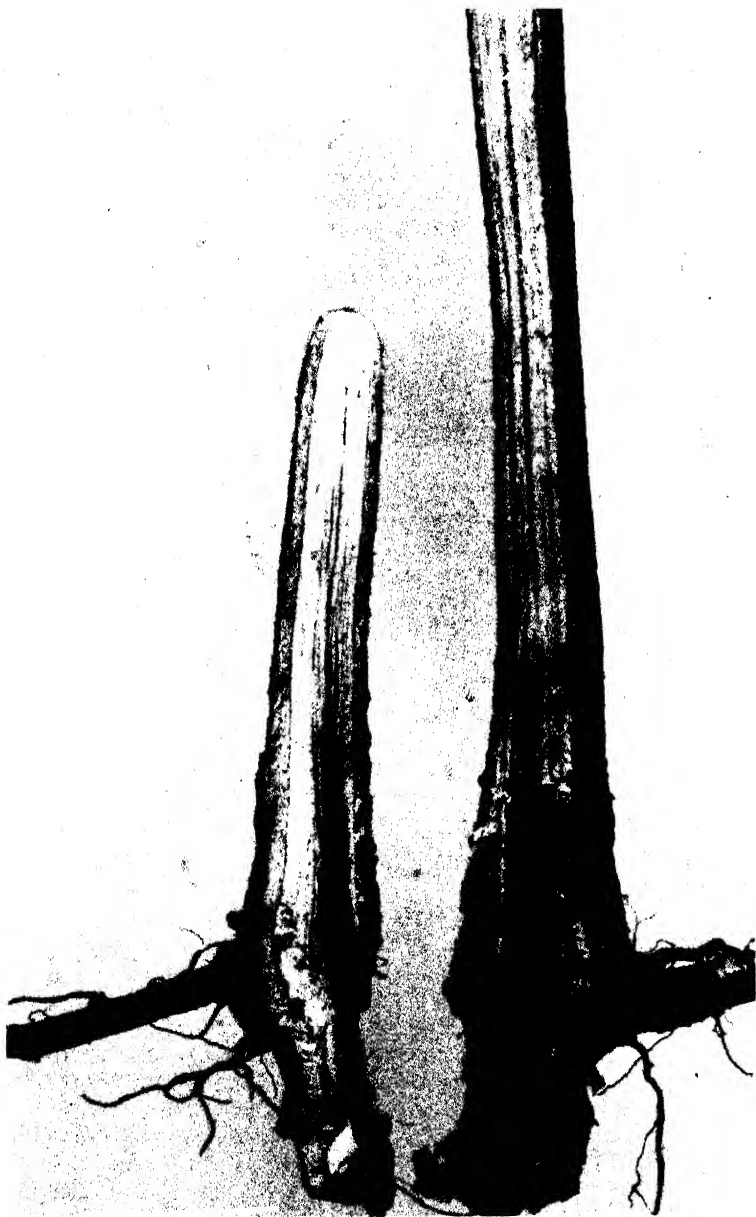


FIGURE 4.—The two halves of a root of a high-carbohydrate cotton plant of experiment 3 that recovered from root rot. There was no evidence that living *Phymatotrichum omnivorum* was present when this plant was removed from the soil 113 days after inoculation and 67 days after nitrogen was added and branches were allowed to develop. Note destruction of taproot by root rot, callusing of the root stump, and development of two strong lateral roots that supported the plant.

The effects of the treatments on the microbial assays of the root-surface saprophytes are reported in a later section of this paper (p. 153).

YOUNG COTTON PLANTS

The nature of the resistance mechanism of young cotton plants to *Phymatotrichum* root rot has been the object of speculation for many years. The experiment reported in this section is one of a number in which the carbohydrate levels of young cotton plants were altered for the purpose of determining whether any relation exists between this factor and susceptibility to root rot.

A difference in the carbohydrate levels between two groups of young plants was effected by the use of (1) Mazda lamps to supplement the midwinter illumination in the greenhouse by about 2,000 foot-candles (at the tops of the plants) over a 14-hour daily period and (2) a cheesecloth shade to reduce the normal greenhouse illumination to about one-third. Between bright and overcast days and between the hours of the day there was an unavoidable wide range in light intensity.

Stoneville 2-B cotton was planted on November 18 in 2-quart mason jars containing a mixture of Houston Black clay and Lufkin fine sandy loam and 300 ml. of Hoagland's solution was added to each jar. The plants were taken to the greenhouse on December 1 and placed in a water bath at 28° C. Supplementary illumination was supplied to all until December 20, when the differential lighting was started. One of the two plants was removed from each jar on January 1 for carbohydrate and nitrogen analyses. On the same date each culture was inoculated with *Phymatotrichum omnivorum* by placing three infected milo seeds at the bottom of a hole 5 cm. deep and 3.0 cm. away from the plant stem. The analytical data reported in table 3 are the means of three samples of five or six plants each. The analyses are reported as percentages of the fresh weight of entire plants.

In this experiment (table 3), a twofold difference in the carbohydrate concentrations of entire 44-day-old plants was without effect on the percentage of plants dying or on the time of death. In similar experiments with yet younger cotton plants, the percentages of plants dying under the two treatments were lower and, as in this experiment, there was no indication that carbohydrate levels affected the incidence of the disease. Direct comparisons between the carbohydrate concentrations found in the entire plants of this experiment and in the root bark of the fruiting-age plants of the preceding experiments are not possible, because of the difference in the plant fractions represented; but, as previously noted, carbohydrate levels tend to be low in young cotton plants. The results of the experiment are regarded as noteworthy in that not all the plants were killed and that the carbohydrate levels exerted no apparent effect upon resistance.

It has been observed by others that cotton (32, 33) and corn (34) seedlings germinated in vitro on agar are attacked by *Phymatotrichum omnivorum*, and the use of substrates rich in carbohydrates has been reported (2, 32, 33, 34) to increase infection. The resistance of young cotton plants has been attributed to their low carbohydrate concentrations (2). The data reported in table 3 fail to disclose any effect upon resistance of changes in carbohydrate levels within young cotton plants. Cotton seedlings germinated on nutrient agar and heavily

overrun by mycelium, with the formation of extensive lesions, have been found by the writers to recover and develop normally when transferred to greenhouse soils.

TABLE 3.—*Effect of carbohydrate levels on the incidence of phymatotrichum root rot in cotton plants 44 days old when inoculated*

[Percentages based on fresh weight of entire plants]

Treatment	Carbohydrates			Nitrogen	Susceptibility to root rot		
	Total sugars	Polysaccharides	Total		Plants surviving	Plants dying	Average period from inoculation to wilting
	Percent	Percent	Percent	Percent	Number	Number	Days
Shaded	0.12	1.46	1.58	0.29	4	12	12.1
Lighted	.78	2.32	3.10	.35	3	15	12.2

The possibility that the resistance of cotton seedlings might be due to an inhibiting substance resulting from photosynthetic activity was considered but was discarded on the basis of observations that followed the inoculation of agar substrates supporting sterile cotton seedlings grown in Erlemeyer flasks (1) exposed to greenhouse light (supplemented by Mazda lamps) and (2) kept in the dark. The exposed and dark-chamber flasks rested on trays in the same constant-temperature bath. The rate of destruction of the seedling tissues by the fungus was relatively slow and essentially equal regardless of whether the plants were exposed to greenhouse light or confined in the dark chamber. This fact is regarded as showing that resistance was not increased by photosynthetic activity. The supporting nutrient-agar substrates employed in the foregoing experiment were made up both with and without calcium, but the calcium was likewise without effect on the severity of the attack.

FIELD EXPERIMENTS

RELATION OF ROOT SPREAD TO ROOT ROT

An important distinction exists between the development of phymatotrichum root rot in potted soils, where each plant is inoculated by some standard procedure, and the spread of the disease from natural infection centers in the field. In the field, the fungus is carried over in resting or active stages from the preceding year and its initial attack on scattered plants results from the meeting of roots and fungus somewhere in the soil mass. The enlargement of root rot spots has been described so often that it requires no elaboration here, except for one fairly obvious point. If it is granted that root rot, after attacking primary plants, spreads to surrounding plants principally by the growth of the fungus along roots and from the roots of one plant to those of neighboring plants, then the abundance, distribution, and overlapping of roots are significant factors in the spread of the disease. The following experiment is believed to illustrate the bearing of root spread upon the spread of root rot.

Three rows of cotton plants were selected in 1941 in a field that had a high root rot mortality during the previous year. When the plants were about 18 inches tall, row 2 was thinned to a spacing of

about 2 feet, which left 122 plants in this row; rows 1 and 3, containing respectively 413 and 438 plants, were not disturbed. On September 15 the percentages of dead cotton in rows 1, 2, and 3 were 68, 33, and 64, respectively. The reduced mortality of the plants in row 2 is believed to have resulted from the reduction in the overlapping and intermingling of the roots of the plants in this row. The thinning of row 2 was performed after the plants were in an advanced floral-bud stage, and the subsequent plant and root development did not compensate for the plants removed. The foregoing situation is in contrast to that existing in spacing experiments in which thinning is carried out in the seedling stage and by midsummer the entire soil space is extensively occupied by roots. In the latter type of experiment, spacing has been found to have little effect on either root rot or yield.

For several reasons it is more difficult to evaluate differences in plant resistance in field experiments than in pot experiments. In the field, values for the period from natural inoculation to wilting are not ascertainable; treatments that affect resistance may also affect spread of the roots and thereby the movement of the fungus from plant to plant. Furthermore, the reduction in population by deaths reduces the chance for a like number of new deaths during a succeeding week. In the field, root rot characteristically appears as islands of dead plants, and the chances of infection of all plants under a treatment are not the same.

The correlation between carbohydrate levels and resistance to root rot as developed in the greenhouse experiments was subjected to further test under field conditions by mutilation and cultural-practice tests conducted in 1941 near Brenham, Tex., on Houston Black clay.

MUTILATION OF COTTON PLANTS

Three plant treatments were employed in the field mutilation experiment as follows: A, Fruited plants with one-half of each leaf cut away; B, normal control plants; C, main-stalk plants (all branches and bolls removed). The plants were grown (1) on land where a high percentage of root rot had occurred during the preceding year and (2) on land of the same soil type one-quarter of a mile distant where no root rot had occurred. The latter plants provided material for the chemical analyses reported in table 4. Both of these experimental plantings were laid out in blocks (15 on the root rot land and 20 on the root-rot-free land) of 15 plants each. Within each block, 5 consecutive plants were taken for each of treatments A, B, and C, the position of these treatments being randomized within the blocks. Stoneville 2-B cotton was planted on May 6 and when about 1 foot tall was thinned to plants about 15 inches apart. The mutilation treatments were started on June 27 and repeated on the same plants once each week thereafter. The first flowers opened during the week ending July 11, and the first deaths from root rot occurred during the week ending July 18.

In the plots on root rot land, cutting away half of each leaf reduced the average number of bolls (not counting those less than 5 days old) on the longest fruiting branch from 1.43 to 0.67, shortened the average length of this branch from 27.6 to 20.9 cm., reduced the average plant height from 67 to 60 cm., and, as indicated by weight of pulled tap-roots in the replicate experiment (table 4), substantially reduced

root development. Reducing leaf area (table 4) had little, if any, effect on the carbohydrate levels in the root bark as determined in duplicate 20-plant samples collected about 1 hour after sunrise on two dates. The fact that the half-leaf treatment markedly retarded plant development and boll setting implies that it also substantially reduced photosynthesis, but a reduction in carbohydrates was not reflected in the early-morning samples.

TABLE 4.—*Effect of mutilation treatments upon the carbohydrate and nitrogen concentrations in root bark and upon root development in cotton plants*

[Percentages expressed on a fresh-weight basis]

Treatment	Collection date	Root-bark carbohydrates					Total nitrogen	Relative weight of pulled taproots
		Total sugars	Starch	Hemi-cellulose	Total	Average for both dates		
		<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	
A. Half leaf	July 31	2.27	0.75	2.96	5.98	5.70	0.14	0.82
	Aug. 14	1.68	.63	3.10	5.41		.15	.78
B. Control	July 31	2.38	.77	2.98	6.13	5.74	.13	1.00
	Aug. 14	1.87	.65	2.83	5.35		.14	1.00
C. Main stalk	July 31	2.64	2.04	2.85	7.53	7.02	.21	1.12
	Aug. 14	2.27	1.40	2.84	6.51		.21	1.49

Limiting plant development to the main stalk (branches and bolls removed, table 4) increased the average plant height from 67 to 108 cm., resulted in enlarged and thickened leaves, and accentuated root development (compare 8). It also substantially increased the concentration of total sugars and more than doubled the concentration of starch in the root bark.

The onset of disease was somewhat more rapid in the control plots (table 5) than in either the half-leaf or main-stalk plots, and this trend was continued to the end of the season. The average of the percentages of remaining live plants that died each week was also somewhat higher in the control plots than in the half-leaf or the main-stalk plots.

TABLE 5.—*Effect of mutilation treatments upon the occurrence of root rot in cotton plants under field conditions*

[The mutilation treatments were started June 27 and repeated once each week thereafter]

Date of count	Plants found dead under indicated treatment		
	Half leaf	Control	Main stalk
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
July 18	1	1	0
July 25	3	8	3
Aug. 1	9	17	5
Aug. 8	12	21	11
Aug. 15	31	39	31
Aug. 22	48	52	36
Aug. 29	49	66	45
Sept. 5	56	68	55
Sept. 12	60	73	60
Deaths weekly, average ¹	9.3	13.3	9.4

¹ Percentage of remaining live plants dying each week.

Differences in carbohydrate levels between the half-leaf and control plants having been negligible, the only explanation of the apparent resistance of the half-leaf plants that has seemed reasonable to the writers is one that concerns the root-spread factor. All plants in each treatment of this experiment were in a single row, and it is possible that the restricted root development of the half-leaf plants retarded the spread of root rot to and among them from plants in the same or in the adjacent untreated rows.

The main-stalk plants had higher carbohydrate concentrations than the control plants, and the indication of retarded development of root rot among these plants is in keeping with the findings on the effects of high carbohydrate levels in the pot experiments. The main-stalk plants had more extensive roots than the control plants, but if these roots tended to increase the spread of root rot it seems evident that this effect was more than offset by the carbohydrate effect.

VARIOUS CULTURAL PRACTICES

A cultural-practice experiment was conducted in the 2 fields that were used for the mutilation experiment. In this factorial-design experiment (4 factors each at 2 levels) the treatments were as follows: (1) 2 plants in hills 14 inches apart versus 2 plants in hills 28 inches apart, (2) plus and minus dusting for the control of boll insects, (3) plus and minus fertilization (100 pounds nitrogen per acre), and (4) plus and minus fall plowing. There were thus 16 treatment combinations. The experiment was replicated in 4 blocks, with each individual plus and minus treatment applied to a total of 32 plots on the root rot land; but on the root-rot-free land, from which the samples were drawn in duplicate for the chemical analyses, there was only a single block of the 16 treatment combinations.

Spacing, dusting, and nitrogen fertilization did not result in statistically significant differences in the percentages of plants killed by root rot, but fall plowing reduced root rot significantly. An average of 25.3 percent of the plants were killed by root rot in the fall-plowed plots, whereas 39.0 percent of the plants were killed in the plots without fall plowing. Notwithstanding the lack of significant effects upon root rot by the spacing, dusting, and nitrogen-fertilization treatments, the changes in root rot resulting from these treatments were found to be correlated with the changes in the sugar concentration of the plants as measured on the root-rot-free land. Fall plowing, which had a large effect on root rot, had no effect upon the carbohydrate concentration. The correlations between total sugars and root rot in both the fall-plowed and non-fall-plowed blocks (considered separately) were in general higher than those between total carbohydrates and root rot. The correlations found between total sugars and root rot are shown in table 6.

The results obtained from this field experiment are consistent with the carbohydrate root rot relations found in the field-mutilation and in the greenhouse experiments. The negative correlation coefficients show that the percentages of dead plants decreased as the carbohydrate concentrations increased.

TABLE 6.—*Correlation between total sugars and percentages of cotton plants dying from phymatotrichum root rot in a factorial experiment*

Dates of observation of indicated correlated items		r^1 obtained for plots—	
Total sugars	Percentage of plants killed	Fall-plowed	Not fall-plowed
July 31	As of Aug. 8	—0.46	—0.51
July 31 and Aug. 15	As of Aug. 15	—0.71	—0.7
Aug. 14	Between Aug. 15 and 22	—0.43	—0.6

¹ r required for significance, —0.50 at 5-percent level and —0.71 at 1-percent level.

ROOT-SURFACE MICROFLORAS AND CARBOHYDRATE LEVELS²

Although conceivably a changed root-bark composition might so affect plant resistance as to be directly responsible for the increased survival periods noted during the course of the foregoing experiments, it is also possible that changes in root-bark carbohydrate concentrations might affect the numbers or types of micro-organisms associated with root surfaces and so allow the operation of antibiotic factors. It has not been shown heretofore that differences in plant carbohydrate levels, such as those here found, produce changes in root-surface microfloras. Half portions of the root bark collected January 27 from plants in greenhouse experiment 3 were subjected to microbiologic examination.

Total numbers of bacteria were enumerated by plate-count procedure on sodium albuminate agar. Bacterial populations (expressed per gram of root-bark material on a moist-weight basis) are shown in table 7. The reduction in total bacterial numbers between the fruited cotton plants (A and B) and the plants without bolls (C and D) were highly significant.

TABLE 7.—*Bacterial numbers associated with roots of cotton plants 21 days after differential treatments (experiment 3) were established*

Treatment	Total carbo- hydrates	Total nitrogen	Blue-green fluorescent bacteria	Spores of aerobic <i>Bacillus</i> spp.	Total bacteria
	Percent	Percent	Number ¹	Number ¹	Number ¹
A. Fruited; normal N	4.45	0.19	166	1,924	926,680
B. Fruited; low N	5.84	.11	222	1,814	733,500
C. Mutilated; normal N	8.34	.47	204	1,389	291,595
D. Mutilated; low N	13.05	.19	901	1,472	384,485

¹ Expressed as thousands per gram of moist root-bark material; each value recorded is the average of determinations on 2 samples.

To determine whether treatment that affected carbohydrate levels resulted in differences in types of bacteria present, two subgroups of bacteria, namely, the blue-green fluorescent *Pseudomonas-Phytomonas* and the aerobic *Bacillus* spp., were enumerated. The fluorescent group, known to be relatively more prevalent on roots than in soil, was determined, by a serial dilution tube method, on a glycerol-asparagine medium (4). Spores of the aerobic *Bacillus* spp., relatively less prevalent on roots than in soil, were determined on nutrient beef agar after pasteurization of root-bark material in dilute aqueous

² By Francis E. Clark and Roland B. Mitchell.

suspensions at 85° C. for 15 minutes. Data for these subgroups are shown in table 7. In general, the number of *Bacillus* spores differed with treatments in much the same manner as did the total bacterial number. Fluorescent bacteria, on the contrary, were least numerous with the lowest carbohydrate content and most numerous with the highest. It would appear, therefore, that both the total bacterial number and the relative prevalence of at least some bacterial subgroups are influenced by the physiological status of the plant.

Some relations between total numbers of bacteria associated with roots and plant susceptibility to root-rotting parasites have been noted previously by others. Working with tobacco plants, Thom and Humfeld (28) noted that varieties susceptible to tobacco root rot showed higher root-surface micropopulations than did nonsusceptible varieties. Similarly, Timonin (29) and Lochhead (19) have found higher numbers of bacteria in the rhizospheres of flax and tobacco varieties susceptible to root rots than in those of rot-resistant varieties. As their results were demonstrated with uninfected plants, Lochhead believed the differences observed were due to inherent differences in physiological function, making, in the case of susceptible plants, conditions somewhat more favorable for general bacterial development. Results of the work here reported suggest that it is possible to produce, within a single variety of cotton, differences in bacterial numbers and root rot susceptibility somewhat analogous to those that exist between different varieties of flax and tobacco.

Whether the reduction in the total number of bacteria and the possible selective encouragement of particular bacterial subgroups following differential treatments are responsible for decreased root rot susceptibility is not answered; however, the fact that the microbial equilibrium is disturbed does suggest that different microbial interrelations or antibiotic phenomena were involved.

ROOT-SURFACE MICROFLORAS AND ROOT ROT RESISTANCE

COTTON AND MAIZE IN STERILE CULTURES

If the micro-organisms that populate the root surfaces of any plant provide protection against a root pathogen, that fact should be most clearly shown by growing the plant on a substrate in which the pathogen is the only micro-organism present. Besides the possibility that certain members of the saprophytic population may actually aid parasitism, the roots of plants growing in sterile cultures should be more quickly or more severely attacked than those of control plants with a normal complement of root saprophytes.

As a means of testing the idea that antibiotic activity of root-surface micro-organisms might be involved in the resistance of certain plants to phymatotrichum root rot, cotton and maize were grown on sterile sand-bentonite substrates. (See p. 140.) Young cotton plants have long been observed to be comparatively resistant to root rot under field conditions, and the immunity of corn was mentioned by Pammel (21) as early as 1888. Taubenhaus and Ezekiel (25), in a study of the resistance of monocotyledons to *Phymatotrichum omnivorum*, were unable to obtain any evidence of infection of corn interplanted with cotton that died following soil inoculations.

The results of all the experiments with the young cotton plants (Stoneville 2-B) were of the same general pattern, whether the substrates were inoculated at the time of planting or after the plants had been in the greenhouse for several weeks. The percentage of plants killed by root rot was always relatively low (ranging from 10 to 30 percent) in both sterile and control cultures. In some experiments a slight advantage seemed to lie with the control cultures and in others with the sterile cultures, but in no experiment was there substantial evidence of significant differences between the two treatments, either in the percentage of plants dying or in the number of days from inoculation to wilting. All the experiments indicated that any protection afforded by the root-surface saprophytes of young cotton

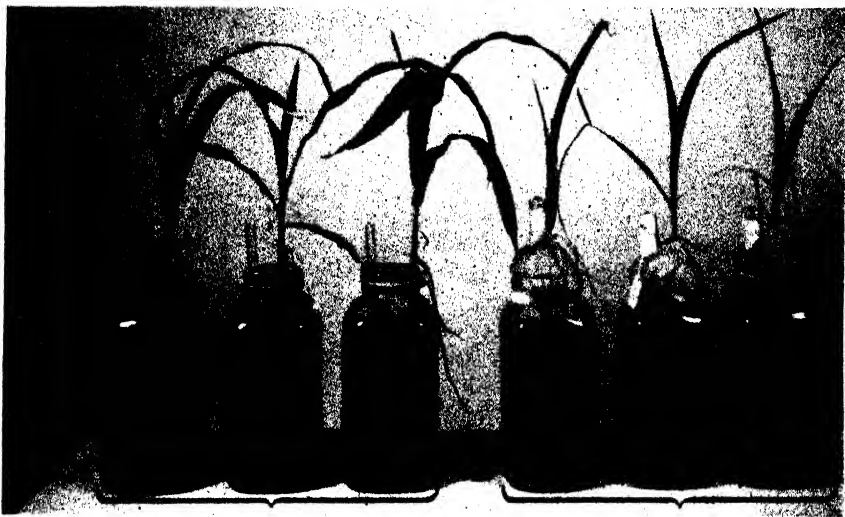


FIGURE 5.—A, Healthy maize plants growing in nonsterile control cultures. B, Dying plants in initially sterile cultures following inoculation with *Phymatotrichum omnivorum*; many infected roots were observed along the walls of the culture vessels.

plants was secondary to chemical or other resistance factors. That the culture methods, the substrate, and the viability and infectivity of the inoculum employed in the investigation were suitable for critical tests is shown by the results of the experiments with maize.

Two experiments were conducted with maize plants. An F_1 hybrid (Kansas Sunflower \times Kansas Yellow Saline) was used in the first experiment and Yellow Surcropper in the second. The procedures followed in both of these tests were the same as those employed in the later and more satisfactory experiments with cotton. The results obtained from each of the two maize experiments were the same and each was conclusive. The plants in the control and sterile cultures grew equally well prior to and for a few days after inoculation with *Phymatotrichum omnivorum*. Beyond that time, however, the maize plants in all the sterile cultures declined rapidly, the leaves wilted and curled (fig. 5), and the plants died. The maize plants on the nonsterile substrates continued for the duration of the experiments to make a normal, healthy growth.

After the inoculations, in the experiments both with cotton and with maize, mycelium and strands of *Phymatotrichum omnivorum* grew out to the walls of the glass jars and developed extensively. This visible growth of the fungus, like the growth of the maize roots, appeared to be equal in the control and sterile cultures. As time progressed the roots of the maize came to be paralleled for long distances and crossed repeatedly by the fungus strands. In this respect there was no difference in the appearance of the control and sterile cultures. In the control cultures, however, maize roots in contact with the fungus remained bright and there was no evidence of any lesions. On the other hand, in the sterile cultures which had no organisms present other than *P. omnivorum*, many lesions developed and long sections of the roots turned brown and died. Furthermore, when the sterile-culture plants were removed from the jars, diseased roots were found throughout the sand mass and the lower portions of the crowns had likewise been attacked. In the nonsterile control cultures no evidence of diseased roots could be found.

The external strands of *Phymatotrichum omnivorum* were removed with sterile forceps from the surface of a number of segments of diseased roots from the sterile cultures. Some of these segments were planted directly on nutrient agar while others were surface-sterilized with 0.1-percent mercuric chloride in 50-percent alcohol and then planted. Growth was obtained on all of these plates, showing that the fungus was present within the roots as well as upon them. The results of these experiments would seem to indicate beyond any reasonable question that the maize plants were killed by the root rot fungus and that the immunity of the maize plant to phymatotrichum root rot is attributable in part, if not entirely, to protection afforded by its root-surface microflora.

Additional evidence that the resistance of seedling cotton plants to *Phymatotrichum omnivorum* may be chemical and that of maize plants primarily antibiotic was provided by other laboratory observations. When cotton seeds were germinated under sterile conditions on agar plates and the substrate was then inoculated with *P. omnivorum*, the attack remained slow and uncertain until a large mass of the fungus had developed. When maize, on the other hand, was similarly germinated, the hypocotyls were freely overrun by the developing fungus and the tissues were rapidly invaded.

The observation that cotton seedlings, after having been overrun and attacked by a mass of *Phymatotrichum omnivorum*, recover when transplanted from nutrient agar to greenhouse soils has a further bearing on the chemical interpretation of resistance. The fungus, when supported in a vigorous condition by an external substrate, produced lesions on the meristematic tissues of the cotton seedlings; but, as viewed in retrospect, it seems probable that the lesions represented only the extracellular activity of the fungus and that the seedling tissue was in itself unsuitable as a substrate for the growth of this fungus.

COMPANION PLANTS

In view of the marked differences known to exist in the number and kind of microbes populating the rhizospheres of different plant species, it seemed worth while to learn whether the resistance of cotton

to *Phymatotrichum omnivorum* might be affected by growing it in immediate proximity to other plants. For the purpose of a test, cotton was planted in the greenhouse in jars of soil in which there were also planted onions, beans, ryegrass, alfalfa, winter peas, vetch, mustard, beets, carrots, and Icele radishes. Following the previously described procedure, *P. omnivorum* was introduced into the soils when the cotton plants were in an advanced floral-bud stage. Only with radish, which has been shown by Clark (4) to have an unusually large number of fluorescent bacteria associated with its roots, was there any suggestion of an increased resistance of the cotton. The effect of radishes on the resistance of cotton was subsequently tested by planting them in the same hills with cotton in short replicated rows at the Temple Substation of the Texas Agricultural Experiment Station. The growth of the radishes was satisfactory under the field conditions, but no effect was observed upon the susceptibility of the cotton plants to root rot. The possibility remains that under other conditions or by the use of other plants some measure of resistance might be gained by growing companion plants in the same hills with cotton, but the chances for success now seem somewhat remote.

DISCUSSION

In each of a series of greenhouse and field experiments with cotton plants of fruiting age, a direct correlation was found between the carbohydrate concentration of the root bark and the resistance of cotton to phymatotrichum root rot. Plants with low carbohydrate concentration usually died within the second week after inoculation, the death of plants with intermediate concentration was substantially delayed, and plants high in carbohydrates remained alive. In all experiments the high-carbohydrate plants suffered initial lesions after inoculation, but the progress of the disease up the taproot was slow and customarily stopped before it reached the uppermost lateral roots. If the plants were maintained in the high-carbohydrate condition for several months, the fungus disappeared from the roots and normal growth was then possible. The complete recovery was probably associated with additional carbohydrate accumulation consequent to the disrupted translocation and to the reduction in water supply caused by the loss of the lower roots.

One cannot conclude from the foregoing data that high carbohydrate concentrations in plant tissues are directly unfavorable to *Phymatotrichum omnivorum* without neglecting the results of extensive laboratory investigations wherein the organism has shown excellent growth in vitro on high-carbohydrate substrates. If *P. omnivorum* were the only organism present when tissues are attacked, its growth should be enhanced rather than retarded by a richer substrate. Under natural conditions, however, saprophytic organisms are also present and the growth of the root rot fungus upon and within plant tissue must in some measure always be competitive. In such systems the growth and survival of *P. omnivorum* on a tissue substrate become dependent upon the relative suitability of the substrate for the various members of the microbial population and upon the interactions between these organisms. In other words, the absence of competition with other organisms differentiates the pure-culture reactions of *P. omnivorum* from those resulting from the ecological relations that exist in natural habitats.

The assays of the root-surface microflora of the cotton plant at four carbohydrate levels showed that the physiological status of this plant has a notable effect on the saprophytic flora of its root surface. Although there were more fluorescent blue-green bacteria, there were fewer *Bacillus* spores and the total number of bacteria was smaller on cotton roots having high carbohydrate concentrations than on those having low carbohydrate concentrations. The fact that the total bacterial number was decreased suggests that the organism or organisms responsible for checking or destroying *Phymatotrichum omnivorum* may also have been unfavorable to other groups. These assays do not show that organisms in themselves were responsible for the suppression and eventual disappearance of *P. omnivorum* from the roots, but they do show that the microbial equilibria were altered with changes in carbohydrate levels, and it is reasonable to assume that without these changes in the microflora *P. omnivorum* would not have been eliminated.

The spread of *Phymatotrichum omnivorum* along the roots of cotton plants low in carbohydrates proceeded with remarkable rapidity, and there was apparently little inhibition to the destruction of the epidermal and underlying tissues. When the plants were high in carbohydrates, on the other hand, the progress of the disease within the tissue mass was slow and eventually stopped. The inhibiting factor, here presumed to be antibiotic, was not limited to the tissue mass but was also a surface effect, inasmuch as the epidermal tissues of high-carbohydrate plants above the lesions remained relatively clean whereas in low-carbohydrate plants epidermal degradation under the mycelial web is fully evident far in advance of the more deeply rotted regions.

Although correlations and circumstantial evidence may lead to reasonable conclusions on the activity of root-surface organisms in the protection of plants against root diseases, such conclusions remain uncertain in the absence of a demonstration that immunity or resistance is lost when the plant is grown on a sterile substrate.

Young cotton plants have long been observed to be resistant to phymatotrichum root rot, and it has been shown (20) that their root-surface microfloral equilibria are notably different from those found on the roots of older cotton plants. Young cotton plants, however, were found to remain resistant to root rot when grown on sterile substrates, and this resistance was not altered when their carbohydrate levels were altered. Circumstantial evidence supports the conclusion that the resistance of high-carbohydrate cotton plants of fruiting age is attributable to antibiotic activity, but a final demonstration that such is the case would necessitate growing plants of this age on sterile substrates. No method of accomplishing this with the tops of the plants freely exposed is known at the present time.

The possible broad significance of the antibiotic factor in the resistance of plants to phymatotrichum root rot was indicated by experiments with maize. Maize plants growing on nonsterile substrates are highly immune to this disease. After inoculations of sterile cultures, however, lesions were rapidly formed on the roots, the leaves wilted and rolled, and the plants died. On the other hand, no lesions were observed on the roots of the maize plants grown on the non-sterile control cultures, even though many roots were paralleled by phymatotrichum strands for long distances along the walls of the

glass culture vessels. The antibiotic protection afforded the maize plant by its root-surface organisms is apparently so great that *Phymatotrichum omnivorum* is unable to exert any effect upon the epidermal tissues.

In almost any field of cotton attacked by root rot, individual plants may be observed that are apparently more resistant to disease than neighboring plants. From such plants (26) and from comparisons between cotton strains, species, and their hybrids (15) efforts have been made to select strains of high resistance. The correlation between resistance and the effects of high carbohydrates, when considered in conjunction with the effects of fruiting and of environmental conditions on carbohydrate concentrations, serves to suggest that apparent resistance may often reflect the effects of physiological status rather than genetic factors. The most productive cotton strains are logically those that most effectively translocate and utilize the products of photosynthesis in boll production. By reason of the low carbohydrate concentrations associated under moist conditions, with high fruitfulness, the productive strains, other conditions being equal, should be the most susceptible to root rot.

Field practices or conditions that increase the productiveness of the cotton plant by increasing fruitfulness and growth, i. e., carbohydrate utilization as distinguished from carbohydrate storage, would also, on the basis of the experiments here reported, increase root rot. However, root rot rarely attacks all parts of a cotton field, and the yield of plants prior to attack is often substantial. Therefore, practices and conditions that increase productiveness, even though associated with treatments that increase the number of dead plants, usually result in net gains in yield.

CONCLUSIONS AND SUMMARY

In potted soils and in the field it was found that the susceptibility of cotton plants of fruiting age to phymatotrichum root rot is related to the carbohydrate content of the root bark. Carbohydrate levels were altered by such means as partial defoliation, defruiting, branch removal, and adjustments in nitrogen supply. With increasing carbohydrate concentration resistance was increased, and plants sufficiently high in carbohydrates withstood attack.

Seedling cotton plants are highly resistant to phymatotrichum root rot, and this resistance was not altered by changes in carbohydrate levels. Likewise, the reactions of seedling cotton plants grown on sterile sand-bentonite substrates showed the resistance to be largely independent of any protection afforded by root-surface saprophytes. A mass attack of *Phymatotrichum omnivorum* produced lesions on seedlings grown on agar substrates, but it seemed doubtful that the living seedling tissues would independently support fungus growth. That the resistance factor is independent of current photosynthetic activity was shown by the similarly weak attack on seedlings growing on agar cultures in flasks kept in a dark chamber and on those exposed to the light.

The correlation between carbohydrate concentration and the resistance of cotton to root rot is believed to reflect antibiotic protection at the higher carbohydrate levels. It was found that the microbial equilibria on the surfaces of cotton roots were markedly altered as the

carbohydrate concentration within the roots was increased. The number of certain organisms tended to increase through successive carbohydrate levels whereas others decreased.

The existence of an important interaction was demonstrated between root-surface saprophytes and the parasitic activity of the root rot fungus *Phymatotrichum omnivorum* by means of experiments with maize. After inoculation with *P. omnivorum* maize plants growing on sterile sand-bentonite substrates were rapidly attacked and killed. The roots of maize plants on otherwise similar but nonsterile substrates remained healthy even though paralleled for long distances by strands of the fungus.

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LOSS OF FURFURAL-YIELDING CONSTITUTENTS FROM WHEAT STRAW ON TREATMENT WITH ALCOHOLIC AND AQUEOUS SODIUM HYDROXIDE SOLUTIONS ¹

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INTRODUCTION

The loss of furfural-yielding constituents in the course of the isolation of hemicelluloses is a matter of considerable importance when the composition of these carbohydrate complexes is to be determined. Obviously, any treatment that results in the removal or degradation of any constituent normally forming a part of the hemicellulose complex would affect the composition of the isolated hemicellulose fraction. The study reported herein was undertaken to determine the extent of loss of furfural-yielding constituents at different stages in the isolation of the hemicelluloses of wheat straw.

REVIEW OF LITERATURE

The various hemicellulose preparations described by the early investigators were contaminated with other organic substances, particularly lignin. Norris and Preece (7),² in their studies on the hemicelluloses of wheat bran, partially delignified the bran by extracting it twice with a boiling 1-percent sodium hydroxide solution in 50-percent ethanol, each extraction being carried out under a reflux condenser for 2 hours.

Norman (5, 6) found that the preliminary delignification of the plant material with hot alcoholic sodium hydroxide solution as used by Norris and Preece (7) resulted in a loss of furfural-yielding constituents.

Buston (3), in preparing the hemicelluloses from cocksfoot grass, treated the plant material with a 1-percent sodium hydroxide solution in 50-percent ethanol and allowed the mixture to digest at room temperature overnight.

Phillips and Davis (8) and Davis and Phillips (4) found that neither the lignin fractions precipitated from the cold alcoholic sodium hydroxide extracts of alfalfa and bagasse nor the filtrates from the lignin fractions yielded any furfural when distilled with 12-percent hydrochloric acid.

Angell and Norris (1) reported that furfural-yielding constituents could not be detected in the hot alcoholic sodium hydroxide extract of hop flowers.

¹ Received for publication July 19, 1944.

² Italic numbers in parentheses refer to Literature Cited, p. 167.

Preece (9) investigated the loss of furfural-yielding constituents at various stages in the process of extracting the hemicelluloses from lilac wood and teak wood. In the case of lilac wood, he found that of the 12.44 percent furfural originally present in the material, a total of 10.66 percent could be accounted for in the ammonium oxalate extract, the alcoholic alkaline extract, the hemicelluloses, and the residual cellulosic material. About 14 percent of the total furfural could not be accounted for. In commenting on this loss Preece (9, *p.* 252) states:

The discrepancy between the two totals [that is between 12.44 and 10.66] is surprisingly high, and it cannot be readily accounted for unless degradative changes have occurred during extraction. It is impossible that as much as 14% of the total furfuraldehyde could be represented by normal "extraction losses."

Preece (9) found that the treatment of unextracted teak wood sawdust with a 4-percent aqueous sodium hydroxide solution at room temperature brought about no loss in the furfural-yielding constituents, although a hot 4-percent aqueous sodium hydroxide solution caused a 3.5 percent loss of furfural. However, teak wood sawdust which had previously been extracted with ether and alcohol and then boiled with a 4-percent aqueous sodium hydroxide solution suffered a loss of 7.2 percent of furfural. Similarly Preece (10) found a loss of furfural-yielding constituents as a result of pretreatment of willow and mahogany woods with boiling sodium hydroxide solutions.

EXPERIMENTAL METHODS

The ground wheat straw used in the experiments was first extracted for 30 hours in a Soxhlet extractor with a 1:2 alcohol-benzene solution and then with a hot (85° C.) 0.5-percent aqueous ammonium oxalate solution until the extract no longer gave a precipitate on the addition of ethanol. The extracted straw was dried in the steam drier.

To 200 gm. of the extracted straw sufficient 2-percent sodium hydroxide solution in 50-percent ethanol was added to immerse the plant material completely. The reaction mixture was stirred manually from time to time and allowed to stand overnight. It was then filtered, the residual material was washed with 70-percent ethanol containing some acetic acid, and the washings were added to the alkaline alcoholic filtrate. This combined filtrate was neutralized with hydrochloric acid and evaporated to dryness on the steam bath. The product obtained was ground, allowed to dry overnight at room temperature, weighed, and the percentage of furfural was determined.

The wheat straw, which had been extracted with the alcoholic sodium hydroxide solution, was dried at room temperature and weighed and a 5-gm. sample was retained for the determination of furfural. To the remainder of the wheat straw, a 4-percent aqueous sodium hydroxide solution was added, in the proportion of 1 part of straw to 15 parts of the alkali solution, the mixture was stirred manually from time to time, and allowed to stand at room temperature for 20 hours. The reaction mixture was filtered, and the previously described extraction with a 4-percent aqueous sodium hydroxide solution was repeated 3 times more, making a total of 4 successive extractions with the alkali solution. The last of these alkaline extracts showed only

a trace of hemicellulose when it was diluted with 3 volumes of 95-percent ethanol. To the combined alkaline extract, 3 volumes of 95-percent ethanol were added, and after the mixture had stood overnight, the hemicelluloses were separated with the aid of the centrifuge. The hemicellulose precipitate was first washed with 70-percent ethanol, acidified with acetic acid, and then washed successively with neutral 70-percent ethanol, 95-percent ethanol, absolute ethanol, and ether. The product was dried in vacuo over sulfuric acid, weighed, and the percentage of furfural determined.

The cellulosic residue (designated as A) from the above treatment with aqueous 4-percent sodium hydroxide solution was washed with 70-percent ethanol acidified with acetic acid, then with neutral 70-percent ethanol, dried in the steam drier and weighed, and a 5-gm. sample was taken for the determination of furfural.

The filtrate and washings from the hemicellulose preparation as well as the washings from the cellulosic residue A were combined and neutralized, and the solution was evaporated to dryness on the steam bath. The residual material on distillation with 12-percent hydrochloric acid yielded no furfural.

Cellulosic residue A was digested with a 2-percent sodium hydroxide solution in 50-percent ethanol under conditions already described. It was then washed with 70-percent ethanol containing some acetic acid, and the washings were added to the alkaline alcoholic filtrate. This solution was neutralized with hydrochloric acid, evaporated to dryness on the steam bath, and the residual material was weighed. When this material was distilled with 12-percent hydrochloric acid, it yielded no furfural.

The cellulosic residue A which had been extracted with the alcoholic sodium hydroxide solution was dried at room temperature, weighed, and a 5-gm. sample was retained for the determination of furfural. The remainder of the cellulosic residue was then extracted four successive times with a 4-percent aqueous sodium hydroxide solution, and the hemicelluloses in the alkaline extract were precipitated, washed, and dried; the procedure already described in connection with the first aqueous alkaline extraction was followed.

The cellulosic residue from the preceding operation (designated as B) was washed with water, and the washings were added to the filtrate from the hemicelluloses of the second 4-percent aqueous sodium hydroxide solution. The washed cellulosic material was first dried in the steam drier, then air-dried, weighed, and a 5-gm. sample was taken for analysis. To the remainder of this material a 4-percent aqueous sodium hydroxide solution was added in the proportion of 1 part of plant material to 15 parts of alkali solution, and the mixture was boiled under a reflux condenser for 1 hour. The reaction mixture was filtered, the residual material was washed with water, and the washings were added to the hot alkaline filtrate. The washed residual material was dried in the steam drier, then air-dried, weighed, and the percentage of furfural was determined.

The hemicelluloses in the extract obtained by boiling with the 4-percent aqueous sodium hydroxide solution were precipitated by following the procedure previously described for the isolation of the hemicelluloses and the treatment of the filtrate and washings for the determination of furfural-yielding constituents. The hemicellulose

precipitates were dried in vacuo at 60° C., weighed, and the percentage of furfural was determined.

All experiments recorded in this paper were made in duplicate, and the results given in table 1 represent the mean of two values.

The furfural determinations were made by the Tollens-Kröber method (2). In determining the furfural balance for each step of the operation, allowance was made for the 5-gm. samples taken for analysis.

RESULTS

The results of the determinations are recorded in table 1.

TABLE 1.—*Loss of furfural-yielding constituents in the isolation of hemicelluloses of wheat straw*

[Furfural in the 200 gm. of starting material=37.64 gm.]

(1) Plant material digested at room temperature for 18 hours with 2-percent sodium hydroxide solution in 50-percent ethanol					(2) Residual material from (1) digested at room temperature 4 successive times with 4-percent aqueous sodium hydroxide solution, each digestion period lasting 20 hours				
Furfural extracted by alcoholic N ₂ O ₂ H		Furfural in residual material		Loss of furfural	Furfural in hemicelluloses		Furfural in residual material		Apparent gain in furfural
Gram	Percent of total	Grams	Percent of total	Percent of total	Grams	Percent of total	Grams	Percent of total	Percent of total
0.66	1.75	34.05	90.46	7.79	22.83	60.65	11.43	30.37	0.56
(3) Residual material from (2) digested at room temperature for 18 hours with 2-percent sodium hydroxide solution in 50-percent ethanol					(4) Residual material from (3) digested at room temperature 4 successive times with 4-percent aqueous sodium hydroxide solution, each digestion period lasting 20 hours				
Furfural extracted by alcoholic N ₂ O ₂ H		Furfural in residual material		Loss of furfural	Furfural in hemicelluloses		Furfural in residual material		Loss of furfural
Gram	Percent of total	Grams	Percent of total	Percent of total	Grams	Percent of total	Grams	Percent of total	Percent of total
0	0	11.32	30.07	0.29	1.45	3.85	8.65	22.98	3.24
(5) Residual material from (4) boiled under reflux condenser for 1 hour with 4-percent aqueous sodium hydroxide solution									
Furfural in hemicelluloses		Furfural in residual material		Apparent gain in furfural					
Grams	Percent of total	Grams	Percent of total	Percent of total					
1.44	3.82	7.50	19.92	0.77					

It will be observed from table 1 that digestion of wheat straw with a 2-percent solution of sodium hydroxide in 50-percent ethanol resulted in a 9.54 percent loss in the yield of furfural of which only 1.75 percent could be accounted for in the alcoholic extract. It would appear, then, that the action of the alcoholic sodium hydroxide solution brought about an actual destruction of some labile furfural-yielding constituent affording 7.79 percent of the total furfural. Moreover, it is rather significant that the greatest loss of furfural-yielding constituents occurred at this stage of the operation; the

losses from the subsequent treatments with alcoholic sodium hydroxide solution and with cold and hot 4-percent aqueous sodium hydroxide solution were much smaller. The gains in the percentages of furfural recorded in sections (2) and (5) of table 1 are rather small and may well have been due to experimental error, although the possibility that some furfural-yielding constituents may have been formed in the course of the treatment with alkali is not excluded.

SUMMARY

A study was made to determine the extent of loss of furfural-yielding constituents at different stages in the isolation of the hemicelluloses of wheat straw.

Partial delignification of the straw with a 2-percent solution of sodium hydroxide in 50-percent ethanol caused a loss of furfural-yielding constituents which amounted to 7.79 percent of the total furfural.

Wheat straw which had been partially delignified with a 2-percent solution of sodium hydroxide in 50-percent ethanol and then subjected to the successive action of cold 4-percent aqueous sodium hydroxide solution, cold 2-percent solution of sodium hydroxide in 50-percent ethanol, cold 4-percent aqueous sodium hydroxide solution, and finally boiling 4-percent aqueous sodium hydroxide solution, suffered little or no loss of furfural-yielding constituents.

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CAROTENE CONTENT OF THE CORN PLANT ¹

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INTRODUCTION

Corn silage ranks with hay as a principal winter roughage for dairy cattle in Wisconsin and adjacent States. It is becoming recognized that content of carotene, the parent substance of vitamin A, is one of the important factors affecting the quality of these winter feeds and of the milk produced thereon. Beginning in 1938, a study was undertaken at this station of the factors that influence the carotene content of hybrid corn (*Zea mays* L.). An initial objective was a determination of the extent to which different inbred lines and the hybrids derived from them varied inherently in capacity to form this substance. On the answer to this question rested the possibility of improving the carotene content of corn by selective breeding.

To this end a large number of genetically pure inbred, single hybrid, and double hybrid strains of corn were raised and their carotene content determined at various stages of growth. During the summer of 1940, for example, approximately 27 inbred, 18 single hybrid, and 30 double hybrid strains were examined. Continuation of the study through four growing seasons has provided data from which the effect of environmental variations, as contrasted with genetic make-up, may be estimated. The effect on carotene content of such factors as the sun-red gene, the sugary gene (sweet corn), the number of plants per hill, and the prevention of pollination were also studied, as was the distribution of the pigment in various parts of the corn plant.

In view of the unique collection of experimental material made available during this work, it was considered advisable to extend the analytical determination to xanthophyll and chlorophyll. The present report, however, is concerned entirely with carotene values, and represents a greatly condensed summary of the complete results which are too extensive to be published in full.³

REVIEW OF LITERATURE

Only a few investigations have been made to determine the variation in carotene content among different strains of a particular plant species. Emsweller, Burrell, and Borthwick (3) ⁴ examined 18 lines

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³ The complete data are available in the doctoral dissertation of John W. Porter, University of Wisconsin, 1942.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 187.

of carrots and found from 32 to 63 mg. of carotene per 100 gm. of dry weight. Later Johnson and Miller (5) analyzed the grain of 19 inbred lines of corn and found that significant variations occurred. They reported a close relationship between the number of genes for yellow endosperm color and the concentration of total carotenoids and β -carotene.

Virtanen (14) reported that the total quantity of carotene in various plants increased rapidly up to the time of flowering and then diminished as the plant matured. Murneek (9) analyzed plants of three species which were grown under long and short day conditions. He found that the short day (reproducing) plants had a higher carotene concentration than the long day (vegetative) plants. Further, analyses of soybean leaves over a portion of the growing season showed a maximum carotene concentration at the time of flowering. Esselen and coworkers (4) found that the vitamin A potency of corn increased as the plant reached full growth, after which there was a considerable decrease. Moon (6) reported a marked loss, after flowering, in the carotene content of grasses. Snyder and Moore (12) analyzed corn leaves from tasseling time to maturity and found the maximum concentration 2 weeks after tasseling. Nagasima (9) reported that the concentration of the carotenoids in seed-bearing plants showed two maxima, the first during the period of most active growth and the second at the time of sexual reproduction.

The effect of season on carotene concentration apparently has not been studied with the exception of determinations of the amount present in pasture grasses during spring, summer, and fall. However, a number of observations on the effect of fertilization, moisture, light intensity, and temperature have been reported. Virtanen (14) found that all factors which have an unfavorable influence on the growth of plants, such as soil acidity, excessive concentrations of phosphate, potassium, sodium chloride, etc., lower the content of carotene in the plant. Also he found that carotene concentration is increased by the application of fertilizers. Pfützer and Pfaff-Limburgerhof (10) found that nitrogen fertilization always increased the carotene content of the plants they studied. Moon (7) reported that nitrogen and potassium each increased the carotene content of pasture grass growing on poor soil. Nitrogen fertilization produced the greatest increase.

Moon (6) reported that drought conditions reduced the carotene concentration of grasses. Atkeson, Peterson, and Aldous (1) found many pasture grasses increased in carotene content following fall rains.

Barnes (2) found that maximum color developed in Chantenay carrots when they were grown at 15° to 21° C. Smith (11) found the carotenoid content of greenhouse-grown tomato fruits to be less than that of the same variety grown out of doors. He reported also that some of the shorter wave lengths of light increase the carotenoid content of fruits.

EXPERIMENTAL METHODS

PREPARATION OF SAMPLE

In preparation for the carotene analyses eight plants of a strain were harvested at the desired stage of development. This number of plants was taken for each sample throughout the study. In 1939 the entire leaf (sheath and blade) was separated from the remainder of

the plant, but in the following 2 years only the leaf blade, minus the midrib, was removed from the rest of the plant. This change was made so that small strain differences could be determined more accurately. Leaf blades are relatively homogeneous tissue which contain 65 to 80 percent of the carotene and only about 10 percent of the total dry weight of the entire plant.

Each year the two fractions were separately chopped into $\frac{1}{8}$ - to $\frac{1}{4}$ -inch pieces with a Gehl ensilage cutter. Chopping rather than grinding was used because, as the values in table 1 show, there is a consider-

TABLE 1.—*Carotene concentration of chopped and ground corn leaf samples*

Description of sample	Method of preparation	
	Chopped ($\frac{1}{8}$ to $\frac{1}{4}$ -inch lengths)	Chopped and then ground (electric meat grinder)
	Micrograms per gram	Micrograms per gram
Greenhouse-grown sweet corn	394	208
	286	264
	227	199
Field-grown sweet corn	611	274
	575	264
Field-grown single hybrid	463	323
		269

able loss of carotene from green corn plants when the latter method is employed.

EXTRACTION OF CAROTENE

After cutting, each fraction was weighed, mixed thoroughly, and duplicate 50-gm. leaf samples and a single 100-gm. sample of the remainder material were taken for carotene analysis. Duplicate 100-gm. samples of each fraction were also taken for moisture determinations. The samples for carotene analyses were immediately introduced into extraction flasks and covered with hot alcohol in order to avoid the rather slight destruction of carotene which was found to occur when cold alcohol was used or when the samples were allowed to stand before the addition of alcohol.

Removal of carotene from the leaf samples was accomplished in three successive extractions by refluxing with 300 to 400-cc. portions of 95-percent ethyl alcohol and decanting the extracts. Samples of the remainder of the plant were extracted three times with 200 to 300-cc. portions of alcohol. Negligible quantities of carotene were removed by further extraction.

STORAGE OF EXTRACTS

The volume of the combined extracts of each sample was measured and a 25-cc. aliquot was taken for carotene and xanthophyll determinations. A 10-cc. aliquot was also taken for chlorophyll determination. The 25-cc. aliquot was introduced into a small bottle (capacity 200 to 250 cc.) and 5 cc. of 10-percent alcohol KOH was added. The solution was saturated with nitrogen, the bottle stoppered, and the sample stored in the dark at -5°C . until analyzed, from 1 to 6 months later. The data in table 2, which refer to longer storage at a higher

temperature, support the belief that there was no loss in carotene content during the storage period.

TABLE 2.—*Stability of carotene during storage of alcoholic extracts of corn*¹

Strain or variety	Analyses performed immediately after extraction	Analyses performed 9 months after extraction
	Micrograms per gram	Micrograms per gram
Golden Glow	70	72
645	79	72
570	61	55
525	63	70
Funk hybrid 235	103	108

¹ The alcoholic extracts were stored under N₂ at room temperature in the dark, and contained KOH.

SEPARATION AND DETERMINATION OF CAROTENE

Carotene and xanthophyll were removed from the alcohol solution by diluting with 30 cc. of water and shaking with 15-cc. portions of Skellysolve B until the latter removed no more color. The combined Skellysolve B fractions were washed once with 30 cc. of water and then extracted with 15-cc. portions of 85-percent ethyl alcohol until the latter came off colorless. The Skellysolve B solution containing the carotene fraction from the leaf material was made to 100 cc. In the case of the stalk material, which was much lower in carotene,

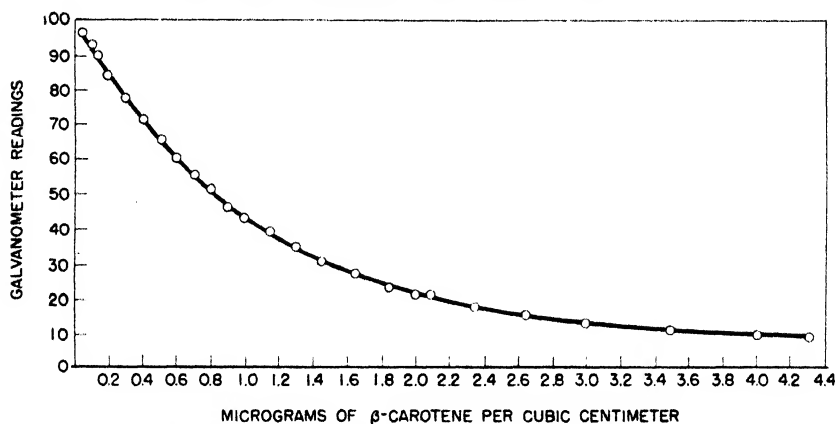


FIGURE 1.—Curve showing absorption of light by β -Carotene in Skellysolve B solution.

the Skellysolve B solution was diluted to 50 cc. The dilution factor for the calculation was therefore 2 instead of 4 as given in the formula below. The pigment content of the Skellysolve B solutions was determined with an Evelyn photoelectric colorimeter equipped with a 440 μ filter. The results were expressed in terms of micrograms of carotene per cubic centimeter by interpolation of the Evelyn readings on a previously constructed standard curve (fig. 1). A Skellysolve B solution of pure β -carotene⁵ was used to obtain this curve.

⁵ Obtained from General Biochemicals, Chagrin Falls, Ohio.

In order to determine whether any carotene was being destroyed during the analysis, Skellysolve B solutions of carotene were allowed to stand exposed to light and air at room temperature for a period of 29 hours. From the data in table 3 it can be seen that there was no loss in carotene during that time.

TABLE 3.—*Stability of carotene in Skellysolve solutions exposed to light and air at room temperature*

Strain or variety	Evelyn galvanometer readings	
	Original value	Value after 29 hours
Golden Glow	82.8	82.8
K ₁₉ ×M ₁₃	72.3	72.3
8-29×A ₁₈	78.3	77.5
14	77.5	78.0
A ₁₈	78.3	78.3
645	84.5	84.5

The amount of carotene per gram of dry matter was calculated by the following formula:

$$\frac{\text{Micrograms of carotene per cc.} \times 4 \times \text{total volume of extract}}{\text{grams of dry weight of the sample}}$$

All the results in the following sections are expressed on the dry basis and unless otherwise stated refer only to leaf material from which the leaf sheath and midrib were removed.

RESULTS

DISTRIBUTION OF CAROTENE IN THE CORN PLANT

To determine the variation in the carotene concentration of different parts of the plant separate analyses were made on the leaves, stalks, husks, and ears of one strain. The values in table 4 show the

TABLE 4.—*Carotene concentration in different parts of the corn plant*

Part of plant ¹	Carotene content at—		Fraction of total dry weight at—	
	Anthesis	Early dent stage	Anthesis	Early dent stage
	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Percent</i>	<i>Percent</i>
Leaves	836	359	9	7
Stalks	53	8	91	93
Husks		10		
Ears		7		

¹ The analyses were made on strain R₁.

concentration of carotene and the percentage of the total dry weight present in these parts of individual plants at two stages of growth. The carotene concentration is so high in the leaves that they contain about two-thirds to three-fourths of the total, although comprising less than one-tenth of the dry weight.

Sprague and Curtis (13) made analyses of different leaves on the same corn plant and reported that carotene decreased rather regularly from the uppermost to the lowest leaves. The writers repeated this experiment with three of their own strains and found the lowest carotene concentration in the uppermost leaf of the plant in two of the three analyses made. The results are shown in table 5.

TABLE 5.—*Carotene concentration of different leaves from the same plant*

Strain	Stage of maturity	Position of leaves ¹	Carotene content
			Micrograms per gram
Single hybrid No. 1.	Late silk	1 and 2	547
		5 and 6	730
		9 and 10	800
Single hybrid No. 2.	Medium milk	1 and 2	474
		5 and 6	615
		8 and 9	559
R ₄	Late silk	2 and 3	593
		4 and 5	663
		8 and 9	572

¹ Counting from top to bottom.

Further analyses were made to determine whether there is a difference in the carotene concentration of the tip and basal portions of the same leaf. The blades of two or three leaves were cut transversely, and the two halves were analyzed. The results, given in table 6, show that the differences were rather slight.

TABLE 6.—*Carotene concentration in tip and basal portions of the same leaf*

Strain	Stage of maturity	Carotene content	
		Tip half	Basal half
		Micrograms per gram	Micrograms per gram
Single hybrid No. 1.	Late silk	613	623
	Medium milk	411	424
Single hybrid No. 2.	Early dent	463	460
	Medium dent	499	477
R ₄	Very early milk	458	374

EFFECT OF GENETIC AND ENVIRONMENTAL FACTORS ON THE CAROTENE CONTENT OF CORN PLANTS

STRAIN DIFFERENCES

In 1940, at the early dent stage of maturity the leaf blades of 25 inbred strains were found to range in carotene concentration from 213 to 544 micrograms per gram and the remainder of the plant from 6 to 24. To determine the statistical significance of such strain differences, analysis of variance tests were performed on the results of analyses made on these strains at anthesis and at the medium dent stage of maturity. Table 7 summarizes the results of the tests and shows that the variance between strains is highly significant at both stages of development.

TABLE 7.—*Analysis of variance table to test the significance of strain differences in carotene concentration of leaf blades*

STAGE OF DEVELOPMENT—ANTHESIS

Variance	Degrees of freedom	Sum of squares	Mean square	F ¹	F (.05) ¹
Within strains	25	12, 214	489		
Between strains	24	560, 940	23, 373	47.8	1.96
Total	49	573, 154			

STAGE OF DEVELOPMENT—MEDIUM DENT

Within strains	27	23, 017	853		
Between strains	26	1, 032, 386	39, 707	46.6	1.92
Total	53	1, 055, 403			

¹ Ratio of variance.

Calculations of the total carotene, dry weight, and carotene concentration per plant of individual strains harvested at the medium dent stage were also made. The results, which are summarized in table 8, show that the strains differed greatly. Similar ranges were found in the other 3 years in which the same analyses were made.

TABLE 8.—*Carotene and dry weight content of inbred, single, and double hybrid strains of corn at the medium dent stage of maturity in 1940*

Strains		Range of carotene per plant	Carotene concentration		Dry weight per plant
Type	Number analyzed		Entire plant	Leaf blades	
		Milligrams	Micrograms per gram	Micrograms per gram	Grams
Inbred	27	3.4-14.1	20.5-75.1	167-503	114-261
Single hybrid	18	7.1-29.2	33.6-73.0	246-529	172-458
Double hybrid	30	9.7-17.5	29.2-76.0	210-690	205-364

STAGE OF MATURITY

In order to learn something of the variation in carotene content of corn plants with age, large plantings of nine strains were made in the summer of 1940, and samples were taken at definite intervals throughout most of the growing season. The results are summarized graphically in figures 2 to 5. Each graph represents average values for the nine strains, except those in figure 2, *A* and *B*, where the values for each strain are shown separately.

The concentration of carotene in the leaves reached a sharp peak at 78 days after planting, when the plants were shedding pollen, but the concentration in the entire plant dropped steadily throughout the period studied. The total carotene content of the entire plant, on the other hand, increased rapidly to a high level at 68 days, and then remained more or less constant until near the end of the season, when it also fell off sharply. The total carotene produced per acre in

comparison with the total yield of silage at different periods of harvest is shown in table 9. It is obvious that large loss of carotene occurs if the harvesting is delayed until the late dent stage.

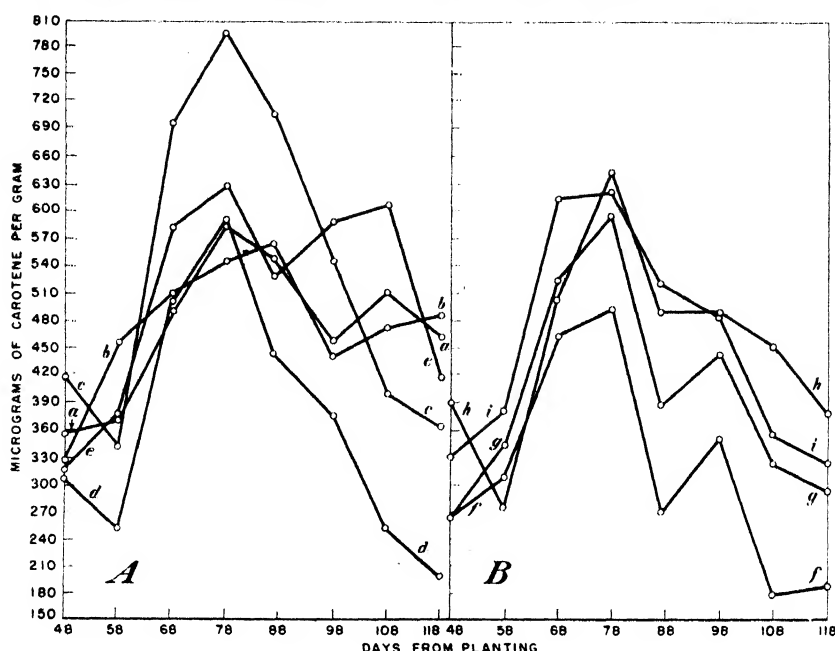


FIGURE 2.—Carotene concentration in the leaf blades of (A) five strains of corn grown in 1940 and (B) four additional strains grown in 1940: *a*, Iowa single hybrid sweet corn; *b*, Country Gentleman sweet corn; *c*, inbred 153; *d*, inbred 3408; *e*, single hybrid 3 x 26; *f*, inbred 6; *g*, inbred 26; *h*, inbred 3; *i*, inbred R19.

TABLE 9.—Yield of carotene and silage at different harvests (1940 season)

Strain or variety	Harvest ¹	Yield per acre	
		Carotene	Silage ²
		Grams	Tons
525.....	First.....	206	10.8
	Second.....	255	12.3
	Third.....	134	12.8
Golden Glow.....	First.....	166	11.1
	Second.....	166	11.3
	Third.....	103	11.1
645.....	First.....	243	12.4
	Second.....	230	14.1
	Third.....	93	14.2
570.....	First.....	174	12.3
	Second.....	169	14.1
	Third.....	99	14.0
696.....	First.....	248	14.7
	Second.....	126	13.2
	Third.....	99	15.2
Funk 235.....	First.....	248	13.8
	Second.....	166	14.9
	Third.....	111	15.7
Average of all strains and varieties.....	First.....	214	12.5
	Second.....	185	13.3
	Third.....	107	13.8

¹ The first harvest was made at the early dent stage, the second at the medium dent stage, and the third at the late dent stage.

² The yields of silage are calculated to a 70-percent moisture basis.

As is well known, strains of corn can be classified according to the number of days required to reach a specific stage of development. When a group of inbred strains was compared on this basis, it was found that with few exceptions those which required the longest time to reach anthesis averaged the most carotene (concentration of leaves and content of the entire plant) and those requiring the shortest period had the least. Those intermediate in carotene content were also intermediate in their growth period requirements. The data are summarized in table 10. It is questionable whether the higher

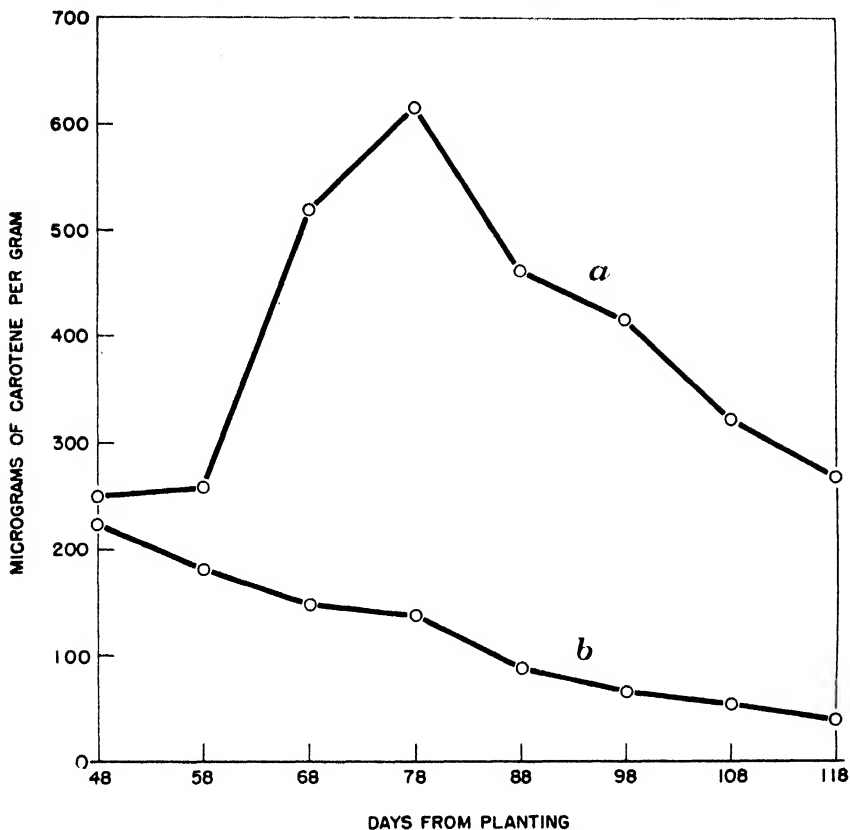


FIGURE 3.—Average carotene concentration of the nine strains for which data are shown in figure 2, A and B: a, Leaf blades; b, entire plant.

carotene content of the slower growing strains is the result of the longer growth period or is due to inherently higher carotene synthesis. The former seems more probable since the carotene concentration of corn leaves increases at a faster rate than the development of the leaf itself. Hence the carotene content of two strains having the same capacity for carotene synthesis would probably be higher in the one requiring the longer growth period to reach anthesis.

That strains differ in their inherent capacity for carotene formation seems fairly certain also, for when several strains which reached the same stage of development at the same time were compared

(table 11), it was found that they differed significantly in carotene content. Here the influence of external factors in producing strain differences was kept at a minimum.

Table 10 also shows the decrease in carotene during the interval from anthesis to the early dent stage. It will be noted that those

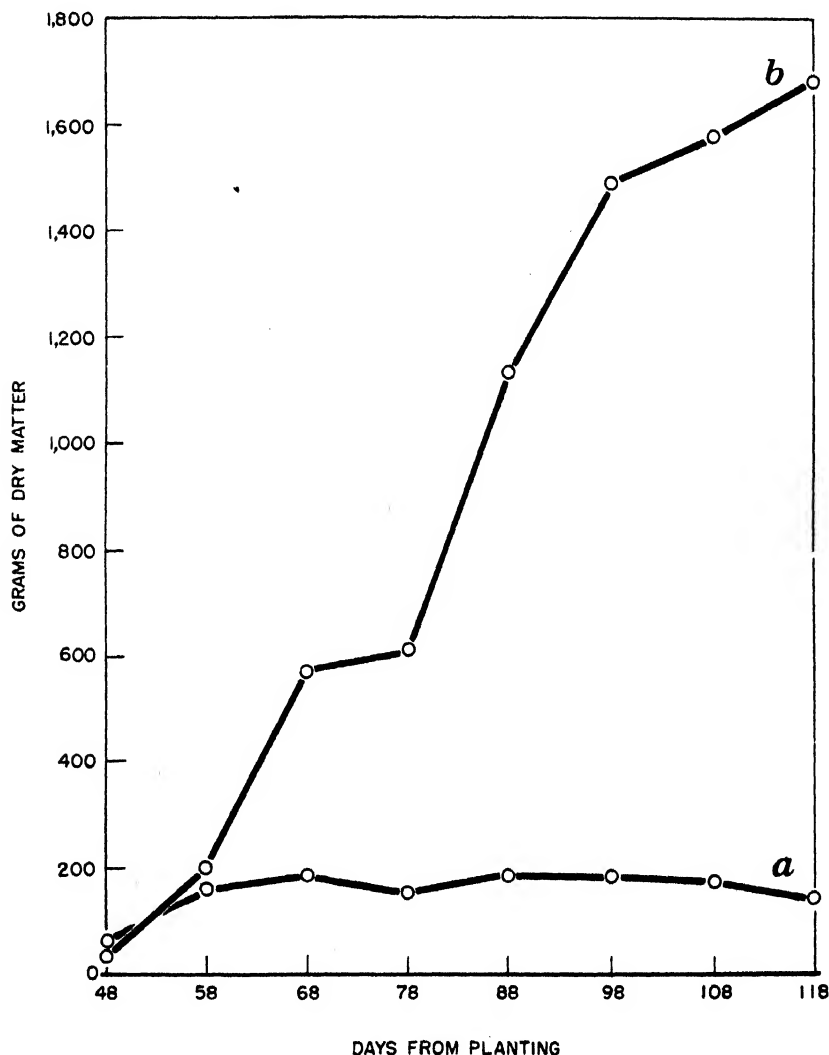


FIGURE 4.—Average dry weight per eight plants of the nine strains for which data are shown in figure 2, *A* and *B*: *a*, Leaf blades; *b*, remainder of plant.

strains requiring the longest growth period lost the most carotene. Whether this loss is correlated with the production of some specific substance in the plant has not been determined. Certainly the decrease in carotene content is not proportional to the dry weight increase of a strain during the same growing period (table 12).

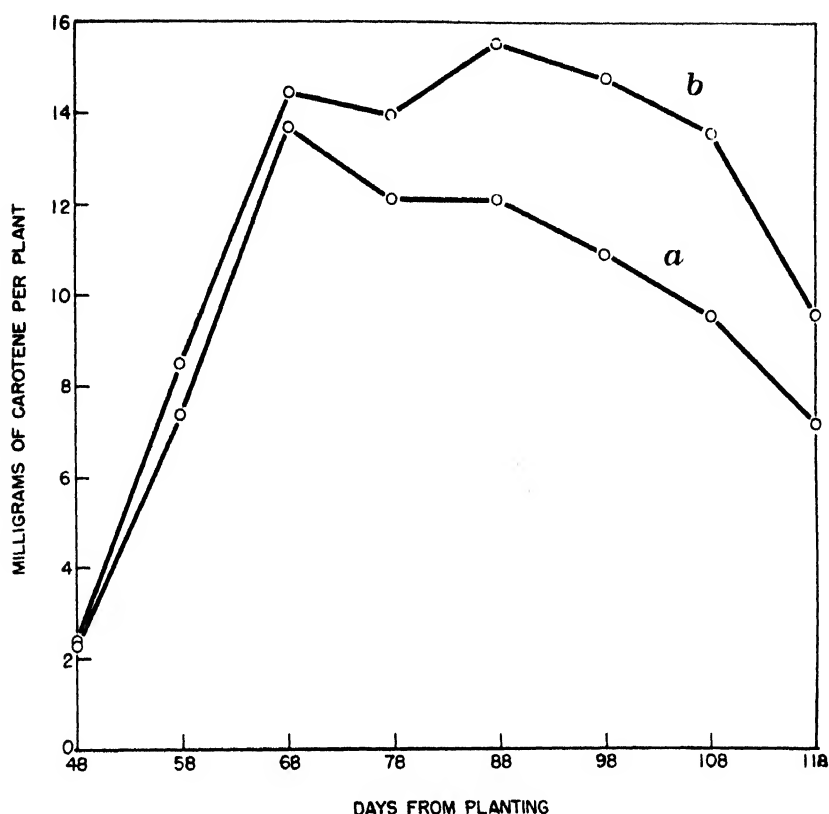


FIGURE 5.—Average total carotene content per plant of the nine strains for which data are shown in figure 2, A and B: a, Leaf blades; b, entire plant.

TABLE 10.—Effect of length of growth period on the carotene concentration of corn

Interval from planting to anthesis (days)	Strains averaged ¹	Carotene in leaf blades at anthesis		Interval from anthesis to early dent stage	Strains averaged ¹	Loss of carotene from leaf blades	
		Range	Average			Range	Average
		Micro-grams per gram	Micro-grams per gram	Days		Micro-grams per gram	Micro-grams per gram
73-77	{25; M ₁₃ ; S. R. M ₁₃ ; 9; S. R. 3.	468-565	523	26-33	{9; S. R. 23; 25; M ₁₃ ; 23.	105-271	177
79-82	{S. R. 26; 8-29; 14; 375; 4362; 23; S. R. 23; R ₃ ; A; 38.	535-736	620	35-40	{S. R. M ₁₃ ; S. R. 3; S. R. 26; 14; R ₃ ; A; A ₆ ; 32; 374; 90; HY; R ₄ ; 4391; 8; 4415.	128-477	281
84	{A ₄₈ ; 32; WF ₈ ; 374; 90; Tr.	535-701	619	42-51	{8-29; 375; 4362; 38; WF ₈ ; Tr; L317B ₂ .	149-574	306
89	{Hy; R ₄ ; L317B ₂ ; 4391; 8; 4415.	618-900	786				

¹ "S. R." denotes sun-red strain.

TABLE 11.—*Differences in carotene formation among strains that reached the same stage of development at the same time (season 1940)*

Strain	Anthesis		Strain	Early dent stage	
	Interval from planting to harvest	Carotene concentration of leaf blades		Interval from planting to harvest	Carotene concentration of leaf blades
	Days	Micrograms per gram		Days	Micrograms per gram
Hy	89	618	32	121	362
		636			372
R ₄	89	837	A ₁₈	121	530
		835			460
8	89	729	4362	121	320
		751			330
4391	89	900	374	122	378
		828			414
L317B ₂	80	854	90	122	396
		890			408
4415	89	792	R ₃	122	267
		760			271
			8-29	124	522
					544
			8	124	520
					484

TABLE 12.—*Decrease in total carotene content of leaves and increase in dry weight of entire plant from pollen-shedding stage to medium dent stage of maturity*

Inbred strain	Carotene decrease per plant	Dry weight increase per plant	Inbred strain	Carotene decrease per plant	Dry weight increase per plant
	Milligrams	Grams		Milligrams	Grams
25	2.29	62	A ₁₈	7.30	95
M ₁₃	3.40	65	32	5.36	121
Sun-red M ₁₃	— .63	55	WF ₂	2.15	147
9	.81	87	374	7.88	87
Sun-red 3	1.29	152	90	.84	74
8-29	4.42	69	Tr	9.18	127
14	6.79	89	Hy	9.14	111
375	— 2.15	130	R ₄	10.30	110
4362	5.21	135	L317B ₂	9.03	150
23	6.49	94	4391	8.95	96
Sun-red 23	5.15	101	8	6.37	106
R ₃	5.05	54	4415	15.00	66
A	6.48	65	Sun-red 26	2.74	86
38	9.75	102			

SEASON

The carotene concentration of the leaves and of the entire plant of a strain and the total carotene content of the plant differed considerably when harvests were made at the same stage of maturity in two successive years (table 13). To test the significance of these differences the results of analyses of the carotene concentration of the leaf blades of five strains at the early, medium, and late dent stages in 1940 and 1941 were subjected to an analysis of variance test. Table 14 shows that the variance between years is highly significant. A similar test was also applied to eight additional strains analyzed at the flowering and medium dent stages for the years 1940 and 1941, with the results shown in table 15. Again it is found that the carotene concentration of the leaf blades differs significantly from year to year. Tables 14 and 15 also show that of the three variables, strain, year, and stage of development, the last named affects the carotene concentration of a plant most. The other two factors are about equal in their effect.

TABLE 13.—*Carotene concentration of corn plants grown in 1940 and 1941 and harvested at the medium dent stage of maturity*

Hybrid or variety	Carotene concentration				Total carotene per plant	
	Leaf blades		Entire plant		1940	1941
	1940 ¹	1941 ¹	1940	1941		
	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Milligrams</i>	<i>Milligrams</i>
525	615; 607	479; 498	76.0	59.5	17.0	15.2
526	480; 536	405; 404	47.7	57.1	11.2	13.6
531	622; 605	445; 499	69.0	39.5	15.7	10.4
606	459; 459	458; 387	58.8	54.8	16.1	13.0
572	690; 572	354; 413	74.4	51.0	17.5	13.5
603	493; 498	340; 332	60.0	35.8	14.6	11.6
Golden Glow	404; 465	381; 434	53.5	46.4	10.9	11.8
Murdock	403; 405	370; 349	50.4	23.5	12.6	5.4
645	544; 580	489; 440	60.5	55.9	15.4	16.6
625	410; 454	428; 405	49.0	36.0	17.2	10.6
640	406; 330	440; 422	45.5	73.1	13.8	16.9
648	400; 368	361; 349	44.5	35.6	13.2	8.8
675	403; 412	304; 311	43.9	29.8	10.0	9.9
676	392; 439	341; 350	45.6	40.2	13.6	10.8
680	360; 327	404; 402	32.7	50.4	10.8	14.9
697	376; 325	388; 431	38.3	32.7	13.9	10.8
Pioneer 322	290; 340	338; 364	30.6	35.2	9.8	9.2
696	398; 408	285; 288	34.9	35.0	10.5	10.9
DeKalb 404A	458; 435	300; 322	43.9	39.9	14.7	10.5
690	382; 347	300; 292	42.4	36.2	12.2	13.1
605	353; 339	358; 358	45.0	42.0	10.4	12.0
Iowa 13	410; 413	386; 488	37.8	44.1	11.0	13.6
Funk 235	330; 387	480; 503	40.5	50.2	12.1	12.7

¹ Each pair of figures represents the results of duplicate analyses.TABLE 14.—*Analysis of variance table showing the significance of seasonal variation in carotene content of corn plants harvested at the same stage of maturity, 1940 and 1941*

Variance	Degrees of freedom	Sums of squares	Mean square	F ¹	F (0.05) ¹
Within strains	30	21,308	710	1	
Between strains	4	89,699	22,425	31.6	2.69
Between harvests	2	282,403	141,202	198.9	3.32
Between years	1	41,870	41,870	59.0	4.17
Strain × harvest interaction	8	49,739	6,217	8.8	2.27
Strain × year interaction	4	58,274	14,569	20.5	2.69
Harvest × year interaction	2	29,908	14,954	21.0	3.32
Strain × harvest × year interaction	8	116,345	14,543	20.5	2.27
Total	59	680,546			

¹ Ratio of variances.TABLE 15.—*Analysis of variance table showing the significance of seasonal variation in carotene content of corn plants at flowering and medium dent stages, 1940 and 1941*

Variance	Degrees of freedom	Sums of squares	Mean square	F ¹	F (0.05) ¹
Within strains	32	32,672	1,021	1	
Between strains	7	308,399	44,057	43.2	2.32
Between harvests	1	1,362,764	1,362,764	1,335.0	4.15
Between years	1	26,122	26,122	25.6	4.15
Strain × harvest interaction	7	67,191	9,599	9.4	2.32
Strain × year interaction	7	26,282	3,740	3.7	2.32
Year × harvest interaction	1	7,679	7,679	7.5	4.15
Strain × year × harvest interaction	7	13,914	1,988	1.9	2.32
Total	63	1,845,023			

¹ Ratio of variances.

PREVENTION OF POLLINATION

Pollination was prevented in several strains by covering ear shoots with paper bags from the time of tasseling until pollinated sister plants in the same row were at the desired stage of maturity. The carotene concentration of the leaves of these pollinated and unpollinated plants is shown in table 16. From the results it is evident that the decrease

TABLE 16.—*Carotene concentration of the entire leaves of pollinated and unpollinated corn plants*

Strain or variety	Carotene concentration at—			
	Early dent stage		Medium dent stage	
	Pollinated	Unpollinated	Pollinated	Unpollinated
		<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>
525.....	¹ 51	¹ 132	101	108
	46	59	110	122
570.....	63	77	74	67
	96	85	109	103
645.....	143	100	111	75
	156	91	135	93
696.....	95	59	88	51
	126	57	111	52
Funk hybrid 235.....	100	96	85	59
	124	120	120	58
Golden Glow.....	¹ 44	¹ 42	119	20
	54	35	96	

¹ Values given are for entire plant.

² These plants were completely lacking in color and were not analyzed.

in carotene concentration of leaves occurs independently of seed formation. Indeed, in some cases the leaf carotene concentration is much lower where pollination was prevented.

SUGARY CHARACTER

It was found that the carotene concentration of the leaves of two sweet corn strains did not decrease in the later stages of development as in dent corn strains. This is clearly shown in figure 6. Otherwise the changes in carotene concentration were similar in the leaves of sweet corn and dent corn strains.

SUN-RED CHARACTER

Sun-red strains of corn contain considerable amounts of anthocyanin pigments whereas dilute sun-red strains have little. By crossing with a sun-red strain and then backcrossing to the original parent it is possible to select a sun-red strain which is almost identical in all other respects with the original non-sun-red (dilute) strain. The carotene concentration of the leaves of four such closely related strains were determined and are reported in table 17. It is apparent that the sun-red character has no effect upon the carotene concentration of corn leaves, for sometimes the sun-red strains had more and at other times less carotene per gram of dry leaf tissue than homologous non-sun-red strains.

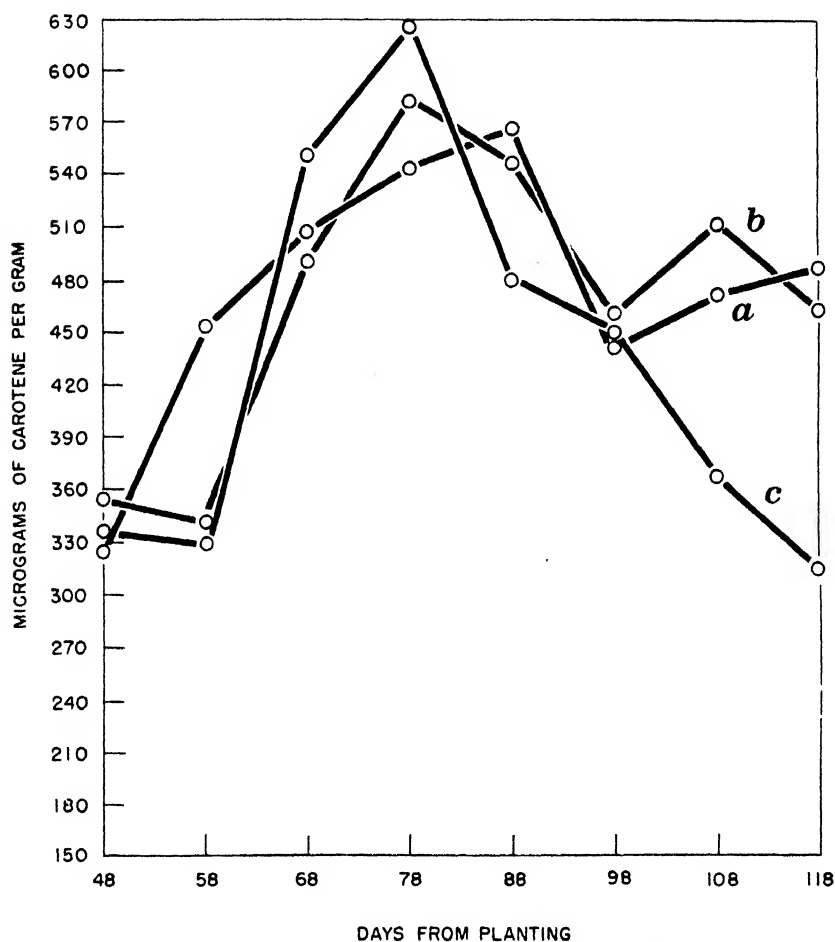


FIGURE 6.—Carotene concentration in the leaf blades of various strains of corn during the 1940 season: *a*, An Iowa single hybrid sweet corn; *b*, Country Gentleman sweet corn; *c*, average concentration of six inbred and one single hybrid dent corn strains.

TABLE 17.—*Carotene concentration of sun-red and non-sun-red strains of corn, 1939 and 1940*

Strain	Carotene concentration in 1939 ¹ at—		Carotene concentration in 1940 ² at—		
	Early dent stage	Medium dent stage	Anthesis	Early dent stage	Medium dent stage
	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Micrograms per gram</i>
Sun red M 13		144	534	334	503
		159	508	330	467
Non sun red M 13		144	526	368	300
		127	565	378	344
Sun red 26	50	50	572	428	378
	72	50	581	399	378
Non sun red 26	46	77	582		283
	60	84	602		296
Sun red 3	127	70	532	405	375
	129	60		403	365
Non sun red 3		65	637		366
		73	640		386
Sun red 23	42	50	625	413	346
	67	51	641	428	306
Non sun red 23	74	85	664	433	288
	113	98	736	425	404

¹ Analyses were made on entire leaf (sheath and blade).² Analyses were made on leaf blade only.

NUMBER OF PLANTS PER HILL

The effect of the number of plants per hill on the carotene content of corn is an important practical question. Table 18 shows the concentration of carotene in the leaf blades and the entire plant and the total carotene content per hill when two, four, and six plants were grown in hills 3 feet apart. The six-plant hills produced over twice as much carotene as those of only two plants.

TABLE 18.—*Effect of number of corn plants per hill on carotene concentration*¹

Strain No.	Plants per hill	Carotene concentration—		Total dry weight per hill of—		Total carotene per hill
		Leaf blades	Entire plant	Leaf blades	Remainder of plant	
		<i>Micrograms per gram</i>	<i>Micrograms per gram</i>	<i>Grams</i>	<i>Grams</i>	<i>Milligrams</i>
625	2	465	40	51	825	35.0
		504				
	4	400	42	111	1220	55.9
		363				
	6	388	55	151	1330	81.5
		443				
696	2	378	33	69	885	31.5
		333				
	4	267	30	108	1190	38.9
		291				
	6	349	35	158	1660	63.6
		327				

¹ Analyses were made on plants at the medium dent stage of development.

RELATION OF CAROTENE CONTENT OF INBRED AND HYBRID STRAINS

It is desirable to know whether one can predict the carotene content of a hybrid from that of its inbred parents. Table 19 presents data which give information on this question. It is evident that a prediction of a hybrid's carotene concentration can be made with only a slight degree of accuracy from the data on the parent inbred strains.

It will also be noted from table 19 that hybrids had a much higher average carotene concentration than inbreds at the medium dent stage. It is not certain whether this is a reflection of the practice of selecting hybrids retaining much of their color at the later stages of maturity or whether it is a definite result of hybridization. Certainly the higher content per plant is largely traceable to the latter factor since a hybrid has a greater weight of leaves per plant than either of its inbred parents.

TABLE 19.—Carotene content of hybrids and their inbred parents

Strains	Carotene concentration of—						Total carotene content per plant	
	Leaves				Entire plant		Hybrids ²	Inbred parents ²
	Hybrids ¹	Inbred parents ¹	Hybrids ²	Inbred parents ²	Hybrids ²	Inbred parents ²		
	Micro-grams per gram	Micro-grams per gram	Micro-grams per gram	Micro-grams per gram	Micro-grams per gram	Micro-grams per gram	Milli-grams	Milli-grams
K ₁₉ ×M ₁₃	586	546; 617	415	352; 322	73.0	351.0; 42.5	12.8	9.7; 5.2
23×26.....	559	700; 592	385	346; 290	50.5	44.1; 39.4	14.1	7.4; 6.8
153×8.....	606	792; 740	308	400; 398	53.4	65.9; 52.5	11.2	9.2; 10.9
22×R ₃	455	776; 632	293	286; 258	46.6	35.5; 38.2	10.2	7.5; 5.2
M ₁₃ ×R ₃	615	546; 632	331	322; 258	48.2	42.5; 38.2	8.7	5.2; 5.2
153×M ₁₃	625	792; 546	461	400; 322	57.9	65.9; 42.5	11.4	9.2; 5.2
K ₁₉ ×153.....	608	792; 617	357	352; 400	48.4	51.0; 65.9	8.3	9.7; 9.2
3×R ₃	623	639; 632	341	376; 258	55.0	38.0; 38.2	11.5	8.6; 5.2
374×A ₄₈	679	683; 661	429	269; 361	47.5	30.4; 52.1	13.9	6.0; 10.2
3×26.....	621	639; 592	468	376; 290	50.4	38.0; 39.4	16.1	8.6; 6.8
38×22.....	553	552; 776	304	180; 286	38.5	20.5; 35.5	16.1	4.1; 7.5
375×R ₃	565	557; 632	253	377; 258	33.6	56.8; 38.2	7.1	9.3; 5.2
W F ₈ ×R ₃	546	553; 632	403	255; 258	54.5	43.6; 38.2	17.4	10.2; 5.2
90×W F ₉	589	596; 553	522	382; 255	66.8	73.3; 43.6	23.4	10.6; 10.2
22×Tr.....	665	776; 622	483	286; 349	56.6	35.5; 53.9	25.9	7.5; 14.1
8-29×A ₄₈	805	719; 661	506	383; 361	72.4	47.4; 52.1	27.4	5.9; 10.2
W F ₈ ×Hy.....	674	553; 627	374	255; 235	47.9	43.6; 29.6	19.2	10.2; 6.8
8-29×Tr.....	789	719; 622	500	383; 349	72.2	47.4; 53.9	29.2	5.9; 14.1

¹ Plants were analyzed at the pollen-shedding stage of development.

² Plants were analyzed at the medium dent stage of development.

³ The first figure in this column refers to the first inbred strain given in column 1; the second figure refers to the other inbred parent.

DISCUSSION

It is evident that a large variety of both hereditary and environmental factors influence the quantity of carotene produced by a particular corn plant. Other things being equal, the total yield of the pigment depends primarily on the stage of maturity at which the crop is harvested. It appears that the optimum time of harvest with respect both to total dry weight and total carotene comes for most strains at the medium dent stage of development, while most of the plants are still reasonably green and succulent. Delay beyond this time results in a large loss of carotene without much compensating increase in total yield of silage (table 9). It may well be also that the nutritive value of the crop with respect to other labile components is higher while the plants are still green.

Since carotenoid pigments normally accumulate in the ear after pollination, it was thought that the great drop in the carotene concentration in the leaves, usually observed following the pollen-shedding stage, might be avoided if pollination were prevented. Such, however, was not the case. The drop in carotene in these experiments was as great or even greater than when pollination was allowed to proceed normally.

At a given stage of maturity most strains were found to differ widely in successive years. The most striking illustration of this fact was provided by the analytical results secured on plants grown in 1939, which on the average contained only about one-half as much carotene as those grown in 1938. The 1940 results were intermediate between these two. Since 1939 was a season of relatively low rainfall at Madison (9.76 inches from May 1 to October 1 vs. 26.1 inches in 1938 and 18.7 in 1940) there is a strong indication that a liberal moisture supply favors high carotene production.

Another environmental factor, namely, the number of plants grown per hill, also was found to have a considerable influence, at least on the total amount of carotene produced on a given area of land (table 18). Although no attempt was made in this work to study such variables as temperature, light intensity, soil fertility, and the like, there is little doubt that these factors likewise have their effect.

Genetic make-up also must be credited with a considerable influence on the carotene content of corn plants. In general, inbred lines, as might be expected, were found to elaborate less carotene than hybrids. Those strains which required a longer time to reach anthesis showed a higher pigment content than more rapidly developing strains. Although the various strains did not remain in the same relative order at the same stage of maturity in successive years, about 75 percent of them remained in the same relation to the average for the entire group at each harvest (i. e., either above or below the average). Furthermore, a few hybrid strains, notably 525, 645, and 531, were found to be consistently among the high carotene groups for 1939, 1940, and 1941. Efforts to correlate the carotene content of various hybrids with that of their inbred parents, however, were not particularly successful (table 19). Likewise the presence or absence of the sun-red gene has almost no effect. In general it seems that the amount of genetic variability with reference to this constituent of the corn plant is not sufficient to justify efforts, for the present at least, towards breeding corn for higher carotene content.

SUMMARY

The concentration of carotene in corn plants is 20 to 50 times higher in the leaf blades than in the remainder of the plant. Stalks, ears, and husks all have about the same concentration, at least in the early dent stage of growth.

Concentration of carotene in the leaves reaches a peak at the pollen-shedding stage and then declines rapidly. Prevention of pollination by bagging the ear shoot did not prevent this drop. The total carotene content of the plant, however, remains relatively constant until near the end of the growing season, when it also falls sharply.

The optimum period for harvesting with respect to total yield of both carotene and silage comes for most strains at the medium dent stage of maturity.

Significant differences in carotene content between stocks, both inbred and hybrid, exist, but are small relative to those associated with stage of development of the plant and with seasonal factors.

Data on two strains suggest that sweet corns may retain a relatively high carotene content as the season advances.

The sun-red gene does not influence the carotene content of corn plants.

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LEAF ELONGATION AND FRUIT GROWTH OF THE DEGLET NOOR DATE IN RELATION TO SOIL-MOISTURE DEFICIENCY¹

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INTRODUCTION

Successful date growing in the United States is confined largely to the desert valleys of California and Arizona, where air temperatures in summer fluctuate from minima of 70° to 90° F. to maxima of 95° to 116° or more. With these high temperatures, together with low relative humidity, soil moisture is rapidly depleted by the date palm (*Phoenix dactylifera* L.) and for successful date production it must be frequently replenished by irrigation. Since the palm does not exhibit any easily observed symptom of water shortage, such as leaf wilting, it frequently suffers from soil-moisture deficiency for many weeks without the grower's knowing it.

For this reason an investigation was begun in 1938 at the United States Date Garden, near Indio, Calif., to find (1) a simple method of detecting when a date palm is being influenced by a deficiency in soil moisture and (2) the extent to which a temporary deficiency in soil moisture may influence fruit size and quality. The systematic measurement of elongation of recently emerged leaves, originally used by Mason (6)³ and later tested to a limited extent by Pillsbury (10), was carefully studied. The details of the technique which was finally developed, together with a discussion of factors other than soil moisture that affect the rate of leaf elongation, have already been reported (2).

The effects of soil-moisture deficiency during the summer upon fruit development in relation to rate of leaf elongation were determined in four experiments, from 1939 to 1941, each experiment in a different planting of bearing Deglet Noor palms. The difficulties involved in obtaining suitable palms with a satisfactory supply and distribution of irrigation water made replications of treatments in any one experiment impracticable. Soil-moisture deficiency was obtained in experimental plots by omitting regular irrigations for a single period of 5 to 10 weeks, but applying at all other times the same irrigation (3 to 9 acre-inches every 10 days) as in the control. In all experiments plot A was the frequently irrigated control and plots B, C, and D had soil-moisture deficiencies in late June and early July,

¹ Received for publication July 26, 1944.

² The writers appreciate the cooperation of H. V. Cavanagh and Kenneth Peck, who kindly furnished parts of their commercial gardens for field plots.

³ Italic numbers in parentheses refer to Literature Cited, p. 200.

in late July and early August, and in late August and early September, respectively. The measurements of average fruit growth and average rate of leaf elongation for each plot were based upon 3 to 6 palms. Fruit growth was measured by determining fresh and dry weights per fruit in weekly collections of 60 to 96 fruits per plot. In order that all fruits might be relatively uniform in development they were selected only from bunches known to have been pollinated within a 14-day period in the spring.

RATE OF PALM LEAF ELONGATION IN RELATION TO SOIL MOISTURE

To substantiate preliminary results obtained in 1938 (7), indicating that the rate of leaf elongation is a sensitive index of the extent of water deficit in the palm, rate of leaf elongation in relation to soil



FIGURE 1.—Ten-year-old Deglet Noor date palms in plot C of the Cavanagh garden on June 4, 1940, being irrigated at the rate of 0.5 cubic foot per second.

moisture was carefully studied in four plots in the Cavanagh garden in 1940. Vigorous 10-year-old palms irrigated by flooding (fig. 1) were used, and one guard row of palms was left between the plots.

The soil, apparently an Indio very fine sand, with no uniform horizons but with strata of silt of varying thicknesses at different depths below the soil surface, is representative of a large proportion of the better date soils. Approximately 3 to 4 acre-inches of water was applied at each irrigation during May and early June and 7 to 9 inches thereafter. Some of this water moved laterally into the unirrigated middle. The average soil moisture per plot was based on a sample of the composite of all soil at each foot depth removed by a soil tube at 12 locations per plot, usually taken the day before and the day after each irrigation. The rate of leaf elongation was based upon measurement of 2 leaves every 2 or 3 days on each of 6 palms per plot.

LEAF ELONGATION WITH FREQUENT IRRIGATION

The frequent irrigation of plot A (control) was representative of commercial plantings using as much as 12 to 14 acre-feet of water annually. After June 25 each irrigation increased the percentage of soil moisture in each of the top 5 feet to more than twice the moisture-equivalent values (table 1). The average percentage moisture content was usually still above the moisture equivalent the day before the next irrigation. The average rate of moisture decrease between irrigations for the top 6 feet, expressed as volume of water, averaged 0.121 acre-inch per day per foot depth of soil, or a total of 7.26 acre-inches in the top 6 feet during 10 days.

The rate of leaf elongation (fig. 2) in plot A between May 10 and October 17 fluctuated between 3.6 and 5.3 cm. per day. The temporarily low rates of elongation during the summer coincided with hot, dry weather and the temporarily high rates with cooler or relatively humid weather. The minima in rates in plot A during hot, dry weather are interpreted as indicating slight water deficits in the palms when transpiration from the leaves exceeded the water supplied by the roots. Minimum rates were frequently followed immediately by relatively high rates, probably resulting from resumption of turgor of enlarging leaf tissue, augmenting normal growth.

TABLE 1.—*Soil-moisture constants and volume weight of soil in plot A (control), Cavanagh garden, 1940*

Soil depth (feet)	Moisture equivalent ¹	Wilting range ²		Volume weight ³
		First permanent wilting point	Ultimate wilting point	
	Percent	Percent	Percent	Grams per cubic centimeter
0-1	7.1	3.0	2.3	1.43
1-2	5.4	2.6	1.9	1.36
2-3	6.7	3.0	1.9	1.39
3-4	4.9	2.2	1.7	1.39
4-5	4.0	2.1	1.5	1.30
5-6	7.0	2.7	2.2	1.22

¹ Determined by J. R. Furr and J. O. Reeve.

² Determined by Furr and Reeve by the method which they have recently described (4).

³ Volume weight (also called apparent specific gravity) is dry weight of soil per unit volume of soil in the field.

Immediately after irrigation of plot A on May 23, June 14, July 23, and September 17, the rate of leaf elongation became appreciably greater than just before irrigation. Similar increases were observed by Pillsbury (9) and Moore and Aldrich (7). Apparently just before irrigation the rate of movement of moisture from the soil into the palm was frequently not sufficient to prevent a slight to moderate water deficit in the palm, but immediately after irrigation the increased water content of the soil in contact with the roots accelerated the rate of water movement into the palm to such an extent that the growth or turgor of cells active in leaf growth was increased. However, for comparison with the appreciable water deficits of palms in plots B, C, and D resulting from the omission of irrigation, the palms in plot A are considered a satisfactory control.

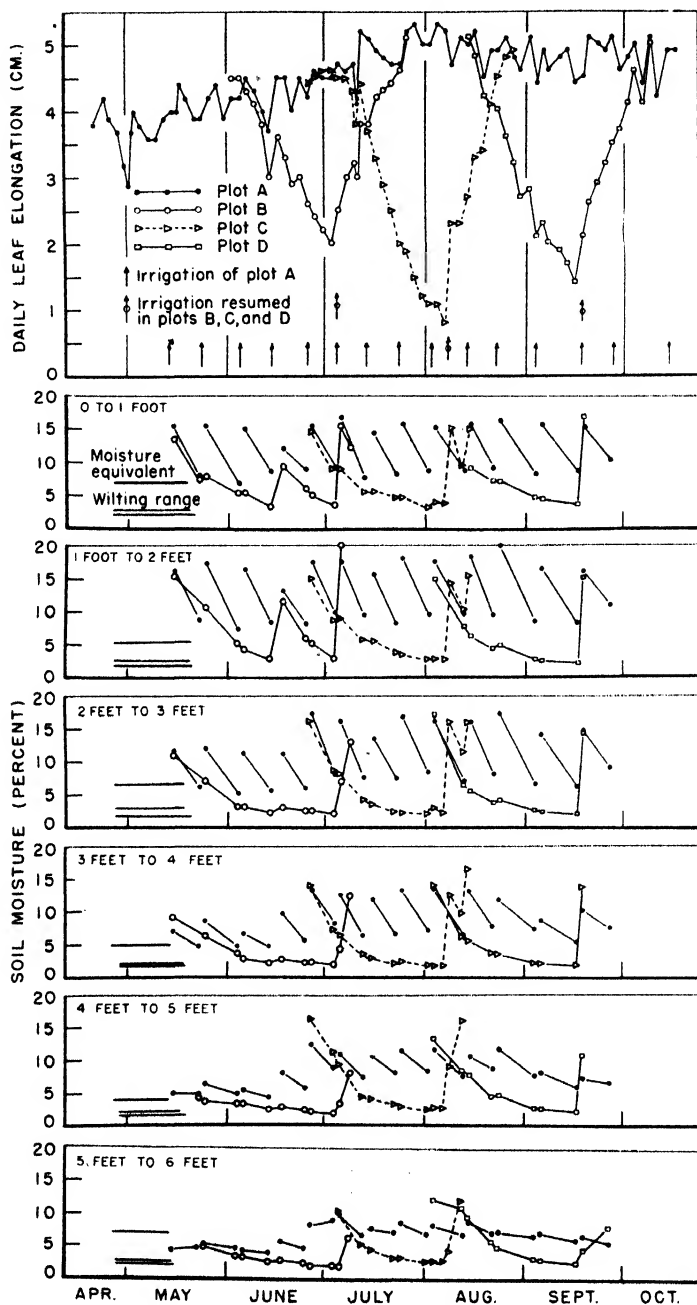


FIGURE 2.—Relation of rate of leaf elongation to average soil-moisture percentages, just after and just before irrigations in plot A (control), and in plots B, C, and D when appreciable water deficits in the date palm were indicated. Plot A was irrigated about every 10 days. In plot B irrigations were omitted from May 14 to July 4, in plot C from June 26 to August 7, and in plot D from August 3 to September 17. The top 2 feet of plot B was accidentally wet on June 14.

LEAF ELONGATION IN RELATION TO SOIL-MOISTURE DEFICIENCY

As stated previously, irrigations were omitted in plot B from May 14 to July 4 (except that the top 2 feet was accidentally wet on June 14), in plot C from June 26 to August 7, and in plot D from August 3 to September 17. The rate of leaf elongation in plots B, C, and D became slower than that in plot A (fig. 2) when the average soil-moisture percentage decreased below the moisture equivalent and approached the wilting range (4) (as determined for the soil in plot A). In general, as the rate of soil-moisture decrease became slower the rate of leaf elongation in plots B, C, and D became progressively lower than that in plot A. By the time the curves for soil moisture had become nearly horizontal, indicating that the soil moisture was in the wilting range and that moisture extraction by the roots was extremely slow, the rate of leaf elongation had decreased below 2 cm. a day. Probably at such times most of the soil moisture that was available to the palm was at depths below 6 feet. These results, together with those obtained earlier (7), indicate that a prolonged decrease in rate of leaf elongation below that of adjacent adequately irrigated palms is evidence of soil-moisture deficiency causing an appreciable water deficit in the palm. Wadsworth (11) obtained somewhat similar results with sugarcane plants.

After the resumption of irrigation in plots B, C, and D the rate of leaf elongation increased, but not to within 0.5 cm. per day of that in plot A until about 2 weeks had elapsed. During these 2 weeks soil moisture must have been available to the roots, so either a delay in water movement into the roots and from there to the crown of the palm or a delay in resumption of division or enlargement of cells at the base of each growing leaf must have been retarding leaf elongation. Such influences retarding leaf elongation will be considered as a part of the appreciable water deficit in the palm resulting from soil-moisture deficiency. Thus a period of appreciable water deficit will be considered as extending from the time when reduced rate of leaf elongation is first detected to the time when the rate is again within 0.5 cm. per day of that of adjacent adequately irrigated palms.

The data relating rate of leaf elongation to soil moisture indicate that equivalent soil-moisture deficiencies (as measured by rate of soil-moisture decrease near or in the wilting range) in late summer and in midsummer, respectively, caused about the same reduction in rate of leaf elongation. Therefore, in experiments where soil moisture was not measured, it has been assumed that equivalent reductions in rate of leaf elongation (as compared with that in the control plots) indicated equivalent soil-moisture deficiencies.

EFFECTS OF APPRECIABLE WATER DEFICIT IN THE PALM

FRUIT DEVELOPMENT

To understand the influence upon fruit development of soil-moisture deficiency at different times during the summer, it was necessary to determine the curves for both fresh- and dry-weight increases of the fruit. Such weight curves were satisfactorily obtained in the previously described Cavanagh plots in 1940, in similar plots in the Cavanagh garden in 1939, in two plots in the Peck garden in 1940, and in somewhat similar plots at the United States Date Garden in 1941. Since the observed effects of appreciable water deficit in the palm

upon the fresh and dry weights of the fruit were essentially the same in each of the experiments, only the curves for the 1940 Cavanagh plots, for which soil-moisture data have already been given, are presented (fig. 3).

The curves in plot A show characteristic changes in the fresh and dry weights of the fruits throughout their growth period. Long (5), who studied the anatomy of fruits from the United States Date Garden experiment in 1941, found the relatively slow increase in fresh weight in late April and early May to be characterized by rapid cell division throughout the fruit and by rapid cell enlargement in the apical and median parts; the rapid fresh-weight increase during June and early July to be due almost entirely to cell enlargement, with cell division only in the basal part of the fruit; and the declining fresh-weight increase during late July and early August to be the result of cell enlargement only in the basal part of the fruit. The decrease in fresh weight per fruit during September is the result of a characteristic decrease in water content of Deglet Noor fruit just before final ripening. The rapid increase in dry weight during August and September is, according to Rygg,⁴ largely the result of sucrose accumulation in the fruit.

The magnitude and duration of the appreciable water deficits in the palm are indicated (fig. 3) by the extent and length of time that the rate of leaf elongation in other plots is below that in plot A. When the rate of leaf elongation in plots B and C, respectively, dropped more than 0.5 cm. per day below that in plot A, the rate of increase in fresh weight of fruits in plots B and C almost immediately dropped below that of fruits in plot A. The rate of dry-weight increase, however, was not immediately reduced proportionally so much as the rate of fresh-weight increase, with the result that the percentage water content of the fruit (data not given) was temporarily reduced. After the resumption of regular irrigation in plots B and C the fruit remained smaller than in plot A, indicating that the reduced increase in fresh weight during the period of appreciable water deficits in the palm must have been largely due to a restriction in the total number or size of cells and not just to a reduction in water content of the fruit.

Delayed effects of the appreciable water deficit in the palm in plots B and C occurred in late summer, after the resumption of irrigation had resulted in about the same rate of leaf elongation in plots B and C as in plot A. The principal delayed effect was the slower rate of dry-weight increase of the fruit in plots B and C than in plot A during late August or early September. This could have been due to the effects of the appreciable water deficit in the palm in reducing the number or size of cells in which the dry matter could accumulate, in reducing the stored carbohydrates (1) available for this dry-matter accumulation, in reducing the efficiency of the vascular bundles for carbohydrate transport, or in modifying the enzymatic or protoplasmic nature of the cells. Associated with the slower rate of dry-weight increase in plots B and C was an approximately 2 weeks' earlier softening of the fruits than in plot A.

An additional delayed effect of appreciable water deficits in the palm in midsummer was observed in plot C in the 1940 experiments

⁴ RYGG, G. L. COMPOSITIONAL CHANGES IN THE DATE FRUIT DURING GROWTH AND RIPENING. U. S. Dept. Agr. Tech. Bul. 910.

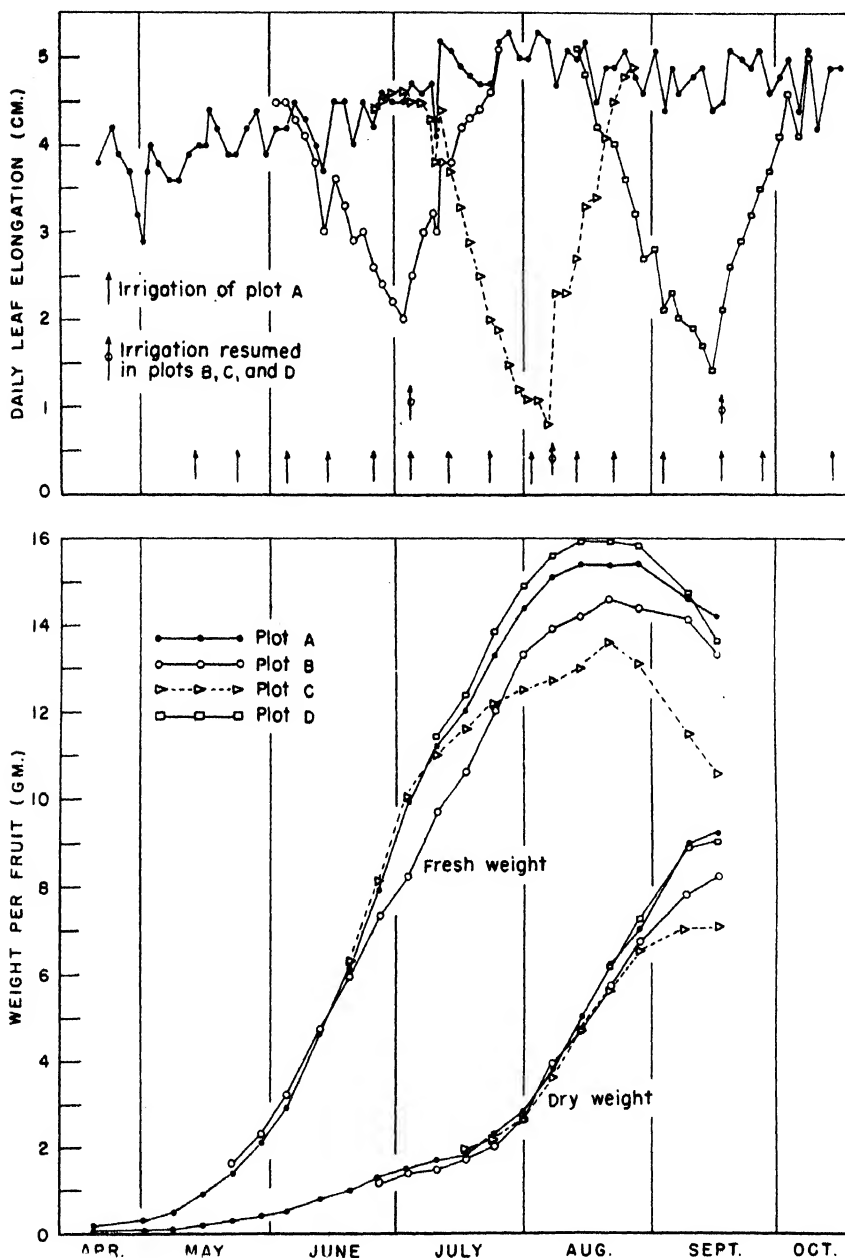


FIGURE 3.—Fresh and dry weights per fruit in relation to appreciable water deficits in the date palm as indicated by rate of leaf elongation, Cavanagh garden, 1940. (Complete curves are presented for plot A, control, but curves for plots B, C, and D are presented only when soil-moisture deficiency caused measurable deviations from curves for plot A.) In plot B irrigation was omitted from May 14 to July 4, in plot C from June 26 to August 7, and in plot D from August 3 to September 17. The top 2 feet of plot B was accidentally wet on June 14.

in both the Cavanagh and Peek gardens. Early in September immature fruits in plot C began to loosen from the perianth and drop, and by the middle of September about 23 percent of the fruits (by weight) had dropped in Cavanagh plot C and about 35 percent in Peek plot C. However, this effect of appreciable water deficits in July did not occur in the 1939 Cavanagh experiment or in the 1941 United States Date Garden experiment.

In plot D, where appreciable water deficits in the palm occurred when cell enlargement had ceased and dry weight (mostly sucrose) was increasing rapidly, the rate of dry-matter accumulation was not reduced below that in plot A, at least in the 1939 and 1940 Cavanagh experiments. Thus, the appreciable water deficits in the palm either did not reduce photosynthesis sufficiently to materially diminish the supply of sugars from the leaves to the fruit, or the utilization of stored carbohydrates compensated for any reduction in the supply of sugars from the leaves. That carbohydrates from stored reserves may be utilized late in the development of the fruit has been suggested by Aldrich and Young (1), whose results show a decrease in the starch content of the trunk during the period of fruit ripening. In the 1941 United States Date Garden experiment, however, with palms just coming into bearing and with such extremely severe water deficits in one of the three palms in plot D that the fruit shriveled before ripening, the indicated rate of dry-matter increase in the fruit dropped considerably below that in plot A. This suggests that when water shortage is sufficiently acute, the ability of the palm to function normally may be so reduced that the rate of dry-matter accumulation in the fruit is greatly lessened. In all three experiments the normal decrease in the total amount of water per fruit during September was slightly greater in plot D than in plot A.

The extent to which the single periods of appreciable water deficit in the palm limited the final weight of the fruit in plots B, C, and D was calculated (table 2). As would be expected from the weight curves previously shown (fig. 2), appreciable water deficits in the palm in June and July (plots B and C) had a much greater effect in reducing the final fruit weight below that of plot A than did the soil-moisture deficiencies in August and September. Although the effects of mid-summer soil-moisture deficiencies in reducing the rates of fresh- and dry-matter increase occurred at different times during the summer, the final fresh and dry weights per fruit were reduced about equally.

TABLE 2.—*Effects of appreciable water deficit in the palm for a single period in limiting the final fresh and dry weights of the fruit*

Location and year of experiment	Fruit weight obtained	Percentage reduction in weight per fruit in each plot below that of fruit in plot A		
		Plot B	Plot C	Plot D
		Percent	Percent	Percent
Cavanagh garden, 1939	{ Fresh ¹	14	12	6
	{ Dry ¹	16	10	0
Cavanagh garden, 1940	{ Fresh ²	6	25	4
	{ Dry ²	11	23	2
Peek garden, 1940	{ Fresh ³		12	
	{ Dry ³		16	
United States Date Garden, 1941	{ Fresh ⁴		16	9
	{ Dry ⁴		13	11

¹ Ripe fruit in second (October 17) picking.

² Partly ripe fruit in September 16 sampling.

³ Ripe fruit in second (September 21) picking

⁴ Ripe fruit in October 9 sampling.

FRUIT QUALITY

Checking, the high-humidity or rain injury to which the fruit is susceptible during June, July, and early August, occurred on fewer fruits in plots B and C than in plot A. These results, which are discussed in a separate report (3), are believed to be due not only to a reduced supply of water from the trunk to the fruit, but also to physical or chemical changes in the fruit brought about by appreciable water deficits in the palm in plots B and C. Blacknose, a shriveling and darkening of the tips of fruits that develop severe checking, seemed to be reduced by the water deficits proportionately more than the checking.

There is a popular belief among date growers that the severe shrivel of ripe fruits often observed in the Deglet Noor variety is most serious when irrigation is not adequate. Although a careful grading of the fruit in each picking from each plot of all experiments showed that appreciable water deficits in the palm were in some cases followed by a slightly increased amount of shrivel in ripe fruits, there was no convincing evidence that shrivel was greatly increased by a single period of soil-moisture deficiency. Since Nixon and Crawford (8) found in fruit-thinning experiments that more shrivel occurred where large numbers of fruits were left on a bunch, it seems probable that shrivel results from a modification of fruit development caused by a limitation in the supply of carbohydrates. Therefore soil-moisture deficiency which is so prolonged as to greatly reduce the vigor of the tree, and perhaps carbohydrates stored in the trunk, might be expected to cause an increase in the number of shriveled fruits.

SUBSEQUENT DEVELOPMENT OF INFLORESCENCES

The effects of the time of appreciable water deficits in the palm in one year upon the number of inflorescences emerging the following spring (table 3) indicate that the deficits in late August and early September (plot D) resulted in fewer inflorescences (statistically significant in two out of three series) the following year than on the control palms. This suggests that reduced photosynthesis during the appreciable water deficits in the palms limited the carbohydrate or other plant foods necessary for development and emergence of the inflorescences.

TABLE 3.—*Effect of time of appreciable water deficit in the palm upon the number of inflorescences appearing per palm the following spring*

Location and year of experiment	Inflorescences per palm, in the spring after appreciable water deficits in the palm			
	Plot A	Plot B	Plot C	Plot D
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
Cavanagh garden, 1939	13.2±0.7	12.8±0.3	11.8±0.5	9.7±0.6
Cavanagh garden, 1940	11.8±0.5	11.8±0.7	12.0±0.3	10.8±0.7
United States Date Garden, 1941	20.7±1.9		17.0±0.6	10.6±0.9

DISCUSSION

These investigations have shown that systematic measurements of the rate of elongation of date leaves provide a satisfactory method for determining when soil moisture is depleted to such an extent as to

reduce the rate of growth of the fruit. The average rate of leaf elongation of recently emerged leaves was fairly constant from late May to October, so that a decrease of 1 cm. or more per day below this normal rate was an indication of soil-moisture deficiency. Fluctuations in rate from day to day during the summer due to variations in weather would tend to smooth out if the calculated rates were based upon intervals of 6 to 7 days between measurements. This normal summer rate of leaf elongation for Deglet Noor palms with adequate soil moisture was found to be between 4 and 5 cm. per day, with the higher normal rate for the palms of greater vigor. Therefore, with Deglet Noor in the summer, a decrease in rate to about 3.5 cm. or less per day would indicate a soil-moisture deficiency. Under circumstances where the rate of leaf elongation appeared to be lower than normal when measurements were started, an increase in rate of about 1 cm. or more per day immediately after an irrigation would indicate that soil-moisture deficiency had existed before the irrigation. If, on the other hand, a rate of 3.5 cm. or less per day were not increased by an irrigation, some other factor, such as a root disease, should be suspected as limiting the growth of the palm.

To extend this method to other seasons of the year or to varieties other than Deglet Noor, it would be necessary to determine the normal rate of leaf elongation on two or three palms irrigated sufficiently often and thoroughly to have the soil moisture adequate at all times. Such a normal rate would be extremely low in the winter (2, 6), would increase gradually during the spring as air and soil temperatures increase, and after a fairly constant rate during the summer would decrease in the fall. With this normal rate as a basis for comparison, a consistent lower rate of leaf elongation on other palms of the same variety would indicate soil-moisture deficiency.

Of particular importance is the fact that, during the period of increase in fresh weight of fruit (late May, June, July, and early August), a prolonged decrease in rate of leaf elongation of 0.5 to 1 cm. or more per day below the normal rate was always accompanied both by a decrease in the rate of growth of the fruit and by a limitation in its final fresh weight. Furthermore, the extent to which the rate of leaf elongation was reduced below the normal rate as a result of soil-moisture deficiency was approximately proportional to the extent to which the rate of fruit growth was reduced. Therefore, when soil-moisture deficiency is suspected of limiting production, the systematic measurement of the rate of leaf elongation can be used to determine when improper irrigation is retarding the development of the fruit.

Although the results indicate that soil-moisture deficiencies in late August and September would not be expected to reduce the final weight of fruit as much as equivalent deficiencies in June or July, it should not be inferred that such late moisture deficiencies are any less detrimental to satisfactory fruit production in the crop of the following year. The evidence that soil-moisture deficiency in late summer resulted in a smaller number of inflorescences the following spring suggests that such deficiency reduces the vigor of the palm.

Since the period of susceptibility to checking coincides with the period of rapid increase in the fresh weight of the fruit, a soil-moisture deficiency of sufficient magnitude to reduce the susceptibility of the

fruit to severe checking during an extended period of high relative humidity or rain would be expected to markedly reduce its rate of growth. This was true in 1940 at both the Cavanagh and Peck gardens. The plots with soil-moisture deficiencies in June or July had lower percentages of blacknose than the controls, but the fresh weight of the ripe fruits from these plots was 6 to 25 percent less than that of the fruits from the controls. Even if no fruit drop had occurred on palms with a soil-moisture deficiency in July, it is doubtful whether the benefits of reduced blacknose would have increased the market value of the fruit sufficiently to compensate for its reduced size and the consequently reduced yield per palm. In years when blacknose is not serious, as in 1939, the reduction in checking alone as a result of soil-moisture deficiency in June and July would certainly not compensate for the reduction in fruit size.

The development of shrivel of ripe fruits appears to be most serious in years with prolonged periods of high temperature, particularly during September. In such years relatively few fruits develop shrivel where soil and cultural conditions have resulted in vigorously growing palms with numerous large leaves, large fruitstalks, and large fruits. Where the leaves, fruitstalks, and fruits are small as a result of unfavorable soil or cultural conditions, adequate irrigation during one season to prevent soil-moisture deficiency did not keep a fairly large percentage of fruits from developing shrivel that season. Thus the role of careful irrigation in reducing the susceptibility of the fruit to shrivel would seem to be the prevention of soil-moisture deficiencies that might hinder the growth of new leaves or reduce the ability of existing leaves to produce carbohydrates. Since carbohydrate reserves probably accumulate in the fall, winter, and spring (1), the prevention of soil-moisture deficiency throughout the year would appear to be desirable.

SUMMARY

The effects of depleted soil moisture on the rate of leaf elongation and fruit development of bearing date palms of the Deglet Noor variety were determined in four series of experimental plots. The moisture content of the soil was measured at 10-day intervals in one series of plots.

The rate of elongation of recently emerged leaves was found to be fairly constant from late May to October.

A decrease in the rate of leaf elongation below that of adjacent frequently irrigated palms was found to be a sensitive index of appreciable water deficits in the palm resulting from soil-moisture deficiency. A prolonged decrease in rate of leaf elongation of 0.5 to 1 cm. or more per day below that of frequently irrigated palms during late May, June, July, and early August, when the fruits were enlarging, was always accompanied by a reduced rate of increase in fresh weight and by limitation in the final fresh weight of the fruits. The rate of increase in dry weight of the fruits did not fall immediately as much as that of fresh weight, so that the percentage water content of the fruits was temporarily reduced. Delayed effects of appreciable water deficits in the palm were manifested in late summer in a reduced rate of increase in fruit dry weight, in approximately 2 weeks' earlier softening of the fruits, and, during one season, in a premature dropping of the

fruits. Equivalent appreciable water deficits in late August and early September, when the fruits were increasing rapidly in dry matter, resulted in a more rapid decrease in their fresh weight during the normal dehydration just prior to ripening, but there was reduced dry-weight accumulation only where the water deficits in the palm were very severe.

Appreciable water deficits in the palm during June, July, or early August, when the fruits are susceptible to the high relative humidity or rain injuries known as checking and blacknose, resulted in fewer fruits developing these injuries than on palms receiving adequate irrigation. Appreciable water deficits in the palms for a single period in the summer did not greatly increase the shrivel of ripe fruits.

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FINAL RESULTS OF THE DUVEL BURIED SEED EXPERIMENT¹

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INTRODUCTION

In 1902 J. W. T. Duvel started a comprehensive experiment at the Arlington Experiment Farm, Rosslyn, Va., to determine the longevity of seeds buried at different depths in the soil under natural conditions. The results after 1 year were reported by Duvel (6),² and the results involving all tests made during the first 20 years of the experiment were reported by Goss (7). Sets of the buried seeds were taken up and tested for viability in the spring of 1932, and again in the fall of 1941, just before the occupation of the Experiment Farm by the United States War Department. The subsequent regrading of the area where the seeds were buried, destroyed the three remaining sets of seeds that it had been intended to test in later years. This report, therefore, completes the record of this experiment on the viability of buried seed.

A number of reports have been published on the germination of seeds of extreme age. J. Ramsbottom, as reported in Nature (1), produced seedlings of *Albizia julibrissin* from seed kept in dry storage for 147 years. Becquerel (3) obtained germination of 158-year-old seed of *Cassia multijuga* Rich.

There have been many published reports on the germination of seeds which circumstantial evidence indicated had been in the soil many years, but it is believed that the first experiment definitely planned to determine the longevity of seeds experimentally buried in the soil was that started by Beal (2) in Michigan in 1879. Darlington (4), in reporting on Beal's experiment after 60 years, found the seeds of 2 and possibly 4 of the 20 buried species surviving. Since the publication by Goss (7) on Duvel's experiment, several publications have appeared on other studies of the longevity of seeds buried in the soil, completed or in progress. Dorph-Petersen (5) mentioned the germination after 18 years of seeds of *Plantago lanceolata* L. buried in soil at the Danish State Seed Testing Station in 1899. He also reported the viability after 6 years of 7 out of 13 species buried at 3 depths in 1904. Goss (8) buried 12 species of weed seeds in California in both irrigated and nonirrigated soil; after 6 years, 5 species were viable from the irrigated and 6 from the nonirrigated soil.

¹ Received for publication August 3, 1944.

² Italic numbers in parentheses refer to Literature Cited, p. 209.

Goss and Brown (9) found that, of the seeds of cultivated and weed rices buried in irrigated and nonirrigated soil in Arkansas, Texas, and California and tested at intervals for 7 years, the weed rices survived longer than the cultivated and longer when the soil was flooded than when it was not. Kjaer (10) found that 20 out of 37 samples of crop and weed seeds germinated better at the end of 5 years when buried in the soil than when held in dry storage. Muenscher (11, p. 6) reported viable seeds at the end of 5 years of all but 3 of 21 kinds of weed seeds buried in clay and in sandy soil at Ithaca, N. Y., in 1928.

MATERIALS AND METHODS

Detailed descriptions of the methods of burial of the seed and of the germination of the seed after removal from the soil have been given by Duvel (6) and by Goss (7). Seeds of 107 species of crop plants and weeds harvested in 1902 (except 2 samples of red clover of the 1900 harvest) were used. Counted seeds of each species, mixed with sterilized soil, were placed in flowerpots, which were then covered with inverted porous flowerpot saucers. An excavation was made with level soil surfaces at 8, 22, and 42 inches below the ground level. Twelve sets of flowerpots containing soil and seeds were placed at each of the deeper levels, and 8 sets were placed at the shallower level. Soil was then carefully packed around the pots, and the excavation was filled to the normal ground level. For the larger seeds, such as



FIGURE 1.—Set of buried seeds at the 42-inch depth, uncovered for removal and germination test in May 1932.

corn, wheat, beans, and cotton, 100 seeds were placed in each pot, but for most kinds 200 seeds were used. The pots ranged in diameter from 2 to 6 inches, depending on the size of the seeds. Sets were removed and tested in 1903, 1905, 1908 (two sets as reported by Goss (7)), 1912, 1918, 1923, 1932, and 1941. The appearance of the covered pots at the 42-inch depth, when uncovered and ready for removal in 1932, is shown in figure 1.

In 1932, seeds from the three depths were removed from the soil on May 4 and promptly tested for germination. The set removed October 11, 1941, represented only the 22- and 42-inch depths, as all the sets at 8 inches had been removed previously. The seeds were tested for viability in sterilized soil in a greenhouse, essentially as described by Goss (7). No tests were discarded until the soil had been examined carefully for sound seeds. Where sound seeds were recovered, they were scarified if they had failed to absorb water or were stratified at a low temperature if they appeared otherwise dormant. It is believed that failure to germinate was not due to lack of suitable germination conditions. When the soil and seeds were being removed from the pots, it was found that ants and earthworms had been active in the soil in some pots, so that some seeds may have been moved or destroyed.

EXPERIMENTAL DATA

The seeds of 15 species of crop plants and 3 species of weeds did not survive even 1 year in the soil. The seeds that never germinated were barley (*Hordeum vulgare* L. [*H. sativum* Jessen]); bean (*Phaseolus vulgaris* L.); buckwheat (*Fagopyrum esculentum* Moench [*F. fagopyrum* (L.) Karst.]); cotton (*Gossypium hirsutum* L.); flax (*Linum usitatissimum* L.); hemp (*Cannabis sativa* L.); maize (*Zea mays* L.), both field and sweet; muskmelon (*Cucumis melo* L.); oats (*Avena sativa* L.); onion (*Allium cepa* L.); pea (*Pisum sativum* L.); rye (*Secale cereale* L.); sunflower, cultivated, (*Helianthus annuus* L.); watermelon (*Citrullus vulgaris* Schrad. [*C. citrullus* (L.) Karst.]); wheat (*Triticum aestivum* L.); chess (*Bromus secalinus* L.); corncockle (*Agrostemma githago* L.); and Russian pigweed (*Aryris amaranthoides* L.). In addition, 8 species germinated so little as to be considered negligible. These were asparagus (*Asparagus officinalis* L.); cabbage (*Brassica oleracea* L.); cowpea (*Vigna cylindrica* (Stickm.) Skeels [*V. catjang* Walp.]); lettuce (*Lactuca sativa* L.); meadow fescue (*Festuca elatior* L.); pepper (*Capsicum frutescens* L. [*C. annum* L.]); scrub pine (*Pinus virginiana* Mill.); and tomato (*Lycopersicon esculentum* Mill. [*L. lycopersicum* L.]). None of these germinated more than 1 percent or more than 1 year with the exception of tomato and lettuce, which germinated 2 different years. Table 1 gives the germination percentages obtained each time the seeds were tested for all kinds except those just listed. To facilitate reference to the previous publications on the experiment, the scientific names used in the original publication by Duvel (6) are given in brackets when they differ from the names now regarded as valid. Half percents have been raised to the next higher percent.

TABLE 1.—Germination in different years of various kinds of seeds buried in 1902

[All kinds of seeds buried except those listed on page 203 as germinating little or none are included; names used by Duvel (6) that differ from present usage appear in brackets]

Family and plant	Depth buried	Seeds germinated in—							
		1903	1905	1908	1912	1918	1923	1932	1941
Poaceae:	Inches	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
<i>Agropyron repens</i> (L.) Beauv. (couch grass)	8	21	0	0	0	0	0	0	0
	22	73	1	0	0	0	0	0	0
	42	67	19	1	2	0	0	0	0
<i>Arena fatua</i> L. (wild oats).....	8	9	0	0	0	0	0	0	0
	22	8	0	0	0	0	0	0	0
	42	18	0	0	0	0	0	0	0
<i>Bromus racemosus</i> L. (upright chess)	8	0	0	12	0	0	0	0	0
	22	0	0	43	0	0	0	0	0
	42	0	0	19	0	0	0	0	0
<i>Elyusine indica</i> (L.) Gaertn. (goosegrass)	8	0	3	1	9	0	0	0	0
	22	0	3	1	0	0	0	0	0
	42	0	3	1	0	0	0	0	0
<i>Elymus canadensis</i> L. (nodding wild-rye)	8	0	1	0	0	0	0	0	0
	22	7	1	0	0	0	0	0	0
	42	22	0	0	0	0	0	0	0
<i>Elymus triticoides</i> Buckl. (wild wheat)	8	2	0	0	0	0	0	0	0
	22	4	0	0	0	0	0	0	0
	42	16	0	0	0	0	0	0	0
<i>Elymus virginicus</i> L. (Virginia wild-rye)	8	2	0	0	0	0	0	0	0
	22	14	0	0	0	0	0	0	0
	42	26	0	0	0	0	0	0	0
<i>Panicum virgatum</i> L. (switchgrass)	8	4	7	0	0	0	0	0	0
	22	9	15	0	0	0	0	0	0
	42	8	15	0	0	0	0	0	0
<i>Phalaris arundinacea</i> L. (reed canary grass)	8	45	40	30	13	12	5	0	0
	22	47	62	53	47	7	12	1	0
	42	57	63	38	16	0	1	0	0
<i>Phleum pratense</i> L. (timothy)	8	0	0	35	23	3	1	0	0
	22	0	0	56	22	2	5	0	0
	42	0	0	47	51	6	13	0	0
<i>Poa pratensis</i> L. (Kentucky bluegrass)	8	16	42	0	17	17	13	1	1
	22	22	66	21	5	27	11	3	1
	42	25	81	47	4	0	19	12	2
<i>Setaria Intescens</i> (Weigel) P. T. Hubb. [<i>Chaetochloa glauca</i> (L.) Scribn.] (yellow foxtail)	8	1	3	6	4	0	0	1	0
	22	1	2	2	5	2	0	0	0
	42	1	2	2	1	0	1	0	0
<i>Setaria verticillata</i> (L.) Beauv. [<i>Chaetochloa verticillata</i> (L.) Scribn.] (foxtail)	8	29	49	40	30	8	7	2	1
	22	36	48	38	35	3	3	0	0
	42	45	62	43	48	1	2	0	1
<i>Setaria viridis</i> (L.) Beauv. [<i>Chaetochloa viridis</i> (L.) Scribn.] (green foxtail)	8	0	56	16	39	29	2	0	0
	22	0	59	13	26	5	6	1	1
	42	0	68	27	80	2	26	0	0
<i>Sporobolus airoides</i> Torr. (hairgrass dropseed)	8	0	0	0	0	0	0	0	0
	22	0	0	7	0	0	5	0	0
	42	0	0	0	8	0	1	0	0
<i>Sporobolus cryptandrus</i> (Torr.) A. Gray (sand dropseed)	8	1	0	1	0	0	0	0	0
	22	2	0	7	0	0	4	0	1
	42	14	0	0	0	0	59	5	0
<i>Sporobolus cryptandrus</i> (Torr.) A. Gray (sand dropseed), hulled seed	8	0	0	0	5	0	0	0	0
	22	0	0	0	8	0	21	5	2
	42	0	0	0	0	0	75	1	0
Cyperaceae:									
<i>Cyperus esculentus</i> L. (yellow nutgrass)	8	0	2	5	7	1	9	0	0
	22	0	3	6	21	5	5	0	0
	42	0	2	0	14	0	17	0	0
Urticaceae:									
<i>Boehmeria nivea</i> (L.) Gaud. (ramie)	8	0	6	0	7	11	39	14	3
	22	0	44	0	10	32	61	14	0
	42	0	54	0	26	32	71	20	6
Polygonaceae:									
<i>Polygonum pensylvanicum</i> L. (smartweed)	8	0	0	0	1	4	0	2	0
	22	0	0	0	3	0	0	1	0
	42	0	0	1	1	0	0	8	0
<i>Polygonum persicaria</i> L. (ladythumb)	8	0	26	0	61	14	1	3	0
	22	0	1	0	32	9	26	9	0
	42	0	0	0	8	1	55	9	0
<i>Polygonum scandens</i> L. (climbing false buckwheat)	8	0	0	0	0	1	0	0	0
	22	0	0	0	0	0	3	0	0
	42	0	0	0	0	0	5	0	1
<i>Rumex crispus</i> L. (curled dock), not cleaned	8	68	69	64	61	43	9	0	0
	22	80	65	67	76	0	24	1	1
	42	79	58	51	73	19	14	12	6

TABLE 1.—Germination in different years of various kinds of seeds buried in 1902—Continued

Family and plant	Depth buried	Seeds germinated in —							
		1903	1905	1908	1912	1918	1923	1932	1941
	Inches	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Polygonaceae—Continued.									
<i>Rumex obtusifolius</i> L. (broad-leaved dock)	8	73	92	89	83	57	59	21	—
	22	73	92	84	86	90	78	33	3
	42	80	94	87	89	84	83	19	5
<i>Rumex salicifolius</i> Weinm. (willow-leaved dock)	8	89	67	33	83	58	0	6	—
	22	86	76	19	62	84	73	46	2
	42	71	68	23	94	89	55	24	6
Chenopodiaceae:									
<i>Beta vulgaris</i> L. (sugar beet)	8	14	0	8	1	0	0	0	—
	22	39	10	35	1	0	1	0	0
	42	40	7	9	4	2	1	0	0
<i>Chenopodium album</i> L. (lambquarters)	8	32	37	1	30	30	0	0	—
	22	64	41	50	37	47	0	24	7
	42	65	51	1	62	47	66	17	9
<i>Chenopodium hybridum</i> L. (maple-leaved goosefoot)	8	8	14	1	37	0	0	0	—
	22	10	30	9	27	15	41	9	0
	42	13	34	42	40	11	0	0	2
Amaranthaceae:									
<i>Amaranthus retroflexus</i> L. (rough pigweed)	8	9	0	1	11	0	0	0	—
	22	11	20	68	36	0	0	0	0
	42	18	12	10	48	0	0	0	0
Phytolaccaceae:									
<i>Phytolacca americana</i> L. (pokeweed)	8	8	66	29	88	0	10	82	—
	22	67	91	69	93	88	75	62	81
	42	81	94	77	77	95	73	76	90
Portulacaceae:									
<i>Portulaca oleracea</i> L. (purslane)	8	39	15	37	22	13	9	1	—
	22	39	31	34	27	20	0	0	0
	42	31	59	11	7	15	38	0	0
Caryophyllaceae [Silenaceae]:									
<i>Saponaria vaccaria</i> L. [<i>Vaccaria vaccaria</i> (L.) Britton] (cowherb)	8	0	0	0	0	0	0	0	—
	22	4	0	1	0	0	0	0	0
	42	7	0	1	28	0	0	0	0
<i>Stellaria media</i> (L.) Cyr. [<i>Alsine media</i> L.] (chickweed)	8	91	65	3	6	0	0	0	—
	22	97	81	47	3	0	0	0	0
	42	93	70	50	22	0	0	0	0
Brassicaceae:									
<i>Brassica nigra</i> (L.) Koch (black mustard)	8	5	27	0	2	0	0	0	—
	22	7	11	1	25	0	38	0	0
	42	7	9	0	26	0	1	0	0
<i>Brassica rapa</i> L. [<i>B. campestris</i> L.] (turnip)	8	0	0	0	0	0	0	0	—
	22	0	0	1	0	0	0	0	0
	42	1	2	1	3	0	0	0	0
<i>Capsella bursa-pastoris</i> (L.) Medik. [<i>Bursa bursa-pastoris</i> (L.) Britton] (shepherdspurse)	8	0	6	0	2	0	0	0	—
	22	0	31	5	6	0	0	0	0
	42	0	28	20	17	47	0	0	0
<i>Erysimum cheiranthoides</i> L. (wormseed)	8	1	0	1	0	0	0	0	—
	22	3	1	0	0	0	0	0	0
	42	4	0	1	0	0	0	0	0
<i>Neslia paniculata</i> (L.) Desv. (ballmustard)	8	23	3	0	10	0	0	0	—
	22	25	6	0	8	0	0	0	0
	42	39	11	3	14	0	0	0	0
<i>Sisymbrium altissimum</i> L. (tall sisymbrium)	8	11	22	79	0	0	0	0	—
	22	18	17	7	3	0	0	0	0
	42	26	49	0	17	0	0	0	0
<i>Thlaspi arvense</i> L. (field pennycress)	8	11	10	34	0	0	0	0	—
	22	8	62	52	46	0	1	11	0
	42	12	31	8	10	0	0	1	0
Rosaceae:									
<i>Potentilla norvegica</i> L. [<i>P. monopetensis</i> L.] (rough cinquefoil)	8	10	90	64	21	47	83	3	—
	22	16	92	63	97	—	69	10	22
	42	22	95	55	89	59	91	7	33
Caesalpiniaceae:									
<i>Cassia maritima</i> L. (wild senna)	8	3	9	14	14	3	1	2	—
	22	3	23	14	7	5	0	0	0
	42	5	25	15	14	3	2	0	0
Fabaceae:									
<i>Lespedeza intermedia</i> (S. Wats.) Britton	8	0	1	0	5	0	2 16	3 1	—
<i>L. frutescens</i> (L.) Britton] (bushclover)	22	0	1	1	3	0	2 34	3 2	2 18
	42	1	2	2	3	0	2 48	3 1	2 32
	8	2	4	1	0	0	0	0	—
<i>Medicago sativa</i> L. (alfalfa)	22	9	1	1	0	0	0	0	0
	42	9	1	0	0	0	0	0	0

See footnotes at end of table.

TABLE 1.—Germination in different years of various kinds of seeds buried in 1902—Continued

Family and plant	Depth buried	Seeds germinated in—							
		1903	1905	1908	1912	1918	1923	1932	1941
	Inches	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Fabaceae—Continued.									
<i>Robinia pseudoacacia</i> L. (black locust)	8	0	5	5	22	20	27	34	
	22	0	1	5	13	6	28	33	30
	42	0	3	4	18	3	31	36	4
<i>Trifolium hybridum</i> L. (alsike clover)	8	2	2	0	3	3	2	0	
	22	4	3	1	2	1	5	2	0
	42	5	2	2	6	4	6	2	0
<i>Trifolium pratense</i> L. (red clover)	8	1	0	0	1	1	3	4	
	22	2	0	0	1	1	4	2	2
	42	2	0	0	3	0	2	4	2
<i>Trifolium pratense</i> L. (red clover), 1900 harvest	8	5	2	1	4	4	4	4	
	22	5	2	2	10		4	2	3
	42	6	6	2	33	1	2	3	3
<i>Trifolium pratense</i> L. (red clover), hard seed from above sample (1900)	8	11	4	4	1	15	16	1	
	22	16	3	7	2		16	5	16
	42	15	5	6	4	1	9	8	12
<i>Trifolium repens</i> L. (white clover)	8	0	0	0	3	3	4	1	
	22	1	0	0	5	0	1	2	0
	42	0	0	1	3	2	0	0	0
Anacardiaceae:									
<i>Rhus glabra</i> L. (smooth sumac)	8	0	0	0	1	0	0	2	
	22	0	0	0	0	0	0	1	2
	42	2	0	0	0	0	0	0	0
Malvaceae:									
<i>Abutilon theophrasti</i> Medik. [<i>A. abutilon</i> L.] (velvetleaf)	8	0	0	45	70	75	70	13	
	22	0	0	25	77	78	62	12	38
	42	0	0	30	72	76	58	6	48
<i>Hibiscus militaris</i> Cav. (halberd-leaved rose-mallow)	8	0	0	38	74	70	58	25	
	22	0	0	67	81	58	36	42	8
	42	0	0	47	74	63	47	47	9
Hypericaceae:									
<i>Ascyrum hypericoides</i> L. (St.-Andrew's-cross)	8	1	0	0	0	0	0	8	
	22	0	0	0	0	0	0	16	0
	42	1	0	0	0	0	0	13	5
Onagraceae:									
<i>Oenothera biennis</i> L. [<i>Onagra biennis</i> (L.) Scop.] (evening-primrose)	8	0	48	0	74	58	61	32	
	22	0	67	0	86	57	88	6	17
	42	0	71	0	90	45	87	59	18
Apiaceae:									
<i>Apium graveolens</i> L. (celery)	8	49	19	1	23	2	0	0	
	22	64	23	4	15	2	11	2	0
	42	60	38	21	32	9	11	1	1
<i>Pastinaca sativa</i> L. (wild parsnip)	8	15	6	15	3	0	0	0	
	22	26	7	11	3	0	0	0	0
	42	32	11	36	14	3	0	0	0
Oleaceae:									
<i>Fraxinus americana</i> L. (white ash)	8	0	0	4	0	0	0	0	
	22	0	0	12	0	0	0	0	0
	42	84	0	4	0	0	0	0	0
Convolvulaceae:									
<i>Convolvulus sepium</i> L. (hedge bindweed)	8	2	20	11	41	47	27	20	
	22	4	11	14	51	66	41	43	34
	42	7	13	21	43	64	43	51	53
<i>Cuscuta epilinum</i> Weihe (flax dodder)	8	16	0	0	0	0	0	0	
	22	24	0	0	0	0	0	0	0
	42	34	0	0	9	0	0	0	0
<i>Cuscuta polygonorum</i> Engelm. (smartweed dodder)	8	12	10	2	16	(6)	(6)	12	
	22	11	16	4	15	(6)	(6)	12	0
	42	13	16	3	10	(6)	(6)	13	1
<i>Ipomoea lacunosa</i> L. (small-flowered white morning-glory)	8	20	80	68	22	6	7	3	
	22	25	94	72	57	71	52	49	31
	42	33	88	92	83	2	57	39	21
Verbenaceae:									
<i>Verbena hastata</i> L. (blue vervain)	8	12	13	0	41	0	83	1	
	22	13	20	0	45	0	71	4	1
	42	14	24	0	26	0	0	2	0
<i>Verbena urticifolia</i> L. (white vervain)	8	24	1	0	19	58	83	1	
	22	25	4	1	24	0	90	46	5
	42	27	0	0	21	64	78	0	1
Solanaceae:									
<i>Datura stramonium</i> L. [<i>D. tatula</i> L.] (jimsonweed)	8	86	41	76	78	24	55	81	
	22	84	38	99	95	82	78	88	91
	42	87	40	91	95	98	56	8	88
<i>Nicotiana tabacum</i> L. (tobacco)	8	47	1	28	40	19	46	13	
	22	70	5	45	50	26	36	8	22
	42	55	19	44	79	29	56	22	17

See footnotes at end of table.

TABLE 1.—Germination in different years of various kinds of seeds buried in 1902—Continued

Family and plant	Depth buried	Seeds germinated in—							
		1903	1905	1908	1912	1918	1923	1932	1941
	Inches	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Solanaceae—Continued.									
<i>Solanum nigrum</i> L. (black nightshade)	8	10	77	31	3	0	82	82	—
	22	11	67	52	81	20	74	42	83
	42	13	63	27	90	29	95	63	79
Scrophulariaceae:									
<i>Verbascum thapsus</i> L. (common mullein)	8	7	18	11	63	38	86	21	—
	22	8	26	8	41	72	90	15	48
	42	26	29	3	83	32	93	57	35
Plantaginaceae:									
<i>Plantago lanceolata</i> L. (buckhorn)	8	21	34	19	4	0	0	0	—
	22	21	51	14	3	0	0	0	0
	42	21	62	17	4	2	0	0	0
<i>Plantago major</i> L. (common plantain)	8	40	68	11	53	18	6	0	—
	22	44	60	10	32	31	14	1	0
	42	47	55	15	41	20	84	0	0
<i>Plantago rugelii</i> Deene. (Rugel's plantain)	8	12	68	4	84	33	0	0	—
	22	12	56	6	75	30	21	0	0
	42	14	66	6	16	24	37	0	0
Cucurbitaceae:									
<i>Cucumis sativus</i> L. (cucumber)	8	0	0	0	0	0	0	0	—
	22	1	0	0	0	0	0	0	0
	42	3	0	0	0	0	0	0	0
Cichoriaceae:									
<i>Lactuca serriola</i> L. [<i>L. scariola</i> L.] (prickly lettuce)	8	64	75	0	0	0	0	0	—
	22	69	59	0	0	0	0	0	0
	42	70	67	0	0	0	0	0	0
<i>Taraxacum laevis</i> (Willd.) DC. [<i>T. erythrospermum</i> Andr.] (red-seeded dandelion)	8	36	29	7	0	0	0	0	—
	22	42	5	1	0	0	0	0	0
	42	46	2	8	0	0	0	0	0
Ambrosiaceae:									
<i>Ambrosia artemisiifolia</i> L. (ragweed)	8	16	13	12	66	69	2	21	—
	22	19	12	6	69	69	84	57	6
	42	21	16	4	62	81	79	10	22
<i>Ambrosia trifida</i> L. (great ragweed)	8	0	0	0	5	0	1	0	—
	22	2	9	0	13	12	15	0	0
	42	6	8	0	6	2	6	0	0
<i>Xanthium pensylvanicum</i> Wallr. (cockle-bur) ¹	8	0	0	0	5	15	0	0	—
	22	0	0	45	5	15	0	0	0
	42	5	0	25	(*)	0	0	0	0
Asteraceae:									
<i>Arcium lappa</i> L. (burdock)	8	43	32	12	53	11	0	0	—
	22	64	58	17	74	34	29	0	0
	42	73	54	26	93	67	17	0	1
<i>Bidens frondosa</i> L. (beggarticks)	8	15	0	53	40	1	0	0	—
	22	17	0	38	64	0	0	0	0
	42	18	0	61	65	0	0	0	0
<i>Chrysanthemum leucanthemum</i> L. (oxeye daisy)	8	21	79	47	78	38	43	4	—
	22	33	79	62	57	37	48	2	1
	42	50	76	82	82	20	39	5	0
<i>Cirsium arvense</i> (L.) Scop. [<i>Carduus arvensis</i> L.] (Canada thistle)	8	21	35	15	6	3	1	0	—
	22	23	29	16	10	1	1	0	0
	42	29	39	26	21	3	5	0	0
<i>Grindelia squarrosa</i> (Pursh) Dunal (gum-plant)	8	31	22	7	4	0	0	0	—
	22	36	11	2	0	0	0	0	0
	42	42	11	5	14	0	0	0	0
<i>Helianthus annuus</i> L. (wild sunflower)	8	44	0	0	0	0	0	0	—
	22	64	0	0	0	0	0	0	0
	42	67	0	0	0	0	0	0	0
<i>Onopordum acanthium</i> L. (Scotch thistle)	8	86	0	82	40	37	37	6	—
	22	93	0	91	72	42	4	53	46
	42	91	0	87	62	32	8	62	30
<i>Rudbeckia hirta</i> L. (black-eyed-susan)	8	7	36	2	53	20	31	12	—
	22	7	41	2	58	49	57	37	0
	42	7	59	3	66	10	52	30	33

¹ 17 percent sound seeds were recovered.² Includes some hard seeds which were recovered and germinated after scarification.³ Hard seeds were not recovered.⁴ In addition, some hard seeds which germinated after scarification were recovered.⁵ In addition, 236 hard seeds were recovered from the 3 depths combined. All germinated after scarification.⁶ Estimated, about 25 percent.⁷ Only 10 fruits with 2 seeds each were buried at each depth.⁸ Record of 25 sprouts evidently an error, perhaps due to root sprouts.

Most viable seeds of the hard-seeded legumes did not germinate until the seed coats were scarified to allow absorption of water, after which germination followed quickly. When dug up in the fall of 1941, the seeds of ragweed (*Ambrosia*) did not germinate under greenhouse conditions but, after the seeds in soil had been kept at 50° F. for about 2 months, germination was prompt.

The seeds of St.-Andrew's-cross (*Ascyrum hypericoides*) showed almost no germination until the test of 1932, when an average of 12 percent of seedlings was obtained from the three depths. In that test the flats were kept for a long period; the first germination of this species occurred approximately 6 months after the seeds were dug up. This delayed germination is in extreme contrast to the behavior of morning-glory (*Ipomoea lacunosa*), seedlings of which appeared within 72 hours after removal from burial in 1932.

For many species there was a gradual or marked decrease of germination in the successive tests; but others, notably pokeweed (*Phytolacca*), jimsonweed (*Datura*), and black nightshade (*Solanum*), maintained a remarkably high germination percentage even after 39 years in the soil.

DISCUSSION

It is worthy of note that the seeds of crop plants having large seeds did not persist even 1 year. The contrast within a species is shown by the sunflower (*Helianthus*); the seed of the cultivated strain did not survive 1 year, whereas 44 to 67 percent of the seeds of the wild sunflower germinated after 1 year although they did not grow thereafter. Even after 39 years there was some viability of some crop plants, including tobacco (*Nicotiana*), red clover (*Trifolium*), celery (*Apium*), and Kentucky bluegrass (*Poa*).

It is probable that the ability of seeds to persist in the soil is connected with the physiological condition known as dormancy. The type of dormancy involved would seem to vary in different species. The seeds of morning-glory (*Ipomoea*) would appear to have been inhibited from germinating by a reduced oxygen supply, as they germinated immediately when brought to the surface, whereas after 39 years in the soil the seeds of ragweed (*Ambrosia*) and St.-Andrew's-cross (*Ascyrum*) required a long period of preparation or special treatment before germination started.

Although the seeds reported as retaining viability in dry storage for long periods have been in general of the hard-seeded type, many of the species reported here as germinating after 39 years in the soil are not hard-seeded but have absorbed moisture while in the soil. While the duration of this buried seed test did not equal the length of time that certain dry seeds have retained their viability, the high germination percentage of seeds of jimsonweed (*Datura*), pokeweed (*Phytolacca*), and black nightshade (*Solanum*), after being buried in the soil for 39 years, indicates the possibility that these species might persist in the soil even longer than the records for seeds in dry storage.

Marked irregularities are apparent in the percentages of germination of the same species from different depths and for succeeding years. These differences may be due both to local differences in environment indirectly brought about by the activity of ants and earthworms already noted and to actual disturbance of the seeds by these organ-

isms. The record of survival of some seeds of a species may be of more significance than the germination percentage. Even though the germination percentages from different levels were often very irregular, the general tendency has been for the seeds from the depth of 8 inches to germinate less than those from the depth of 42 inches. Of the total number of seedlings obtained from 1903 through 1932, approximately 27 percent were from the 8-inch, 34 percent from the 22-inch, and 39 percent from the 42-inch level. This proportion did not vary greatly for the different years.

SUMMARY

In tests to determine the viability of buried seeds it was found that of 107 species buried in 1902, 71 grew after 1 year, 61 grew after 3 years, 68 grew after 6 years, 68 grew after 10 years, 51 grew after 16 years, 51 grew after 20 years, 44 grew after 30 years, and 36 grew after 39 years.

The following 10 species that grew in 1923 failed to grow in 1932: *Ambrosia trifida*, *Arctium lappa*, *Beta vulgaris*, *Brassica nigra*, *Cirsium arvense*, *Cyperus esculentus*, *Phleum pratense*, *Plantago rugelii*, *Polygonum scandens*, and *Sporobolus airoides*. Of these, *A. lappa* and *P. scandens* showed some life again in 1941, and 3 species that grew in 1932 (*Ascyrum hypericoides*, *Polygonum pensylvanicum*, and *Rhus glabra*) had failed to germinate in 1923.

The following 10 species that grew in 1932 failed to germinate in 1941: *Cassia marilandica*, *Phalaris arundinacea*, *Plantago major*, *Polygonum pensylvanicum*, *P. persicaria*, *Portulaca oleracea*, *Setaria lutescens*, *Thlaspi arvense*, *Trifolium hybridum*, and *T. repens*.

The 16 species, representing 10 plant families, having the highest percentage of germination in 1941 (more than 15 percent from at least one depth) were *Abutilon theophrasti*, *Ambrosia artemisiifolia*, *Convolvulus sepium*, *Datura stramonium*, *Ipomoea lacunosa*, *Lespedeza intermedia*, *Nicotiana tabacum*, *Oenothera biennis*, *Onopordum acanthium*, *Phytolacca americana*, *Potentilla norvegica*, *Robinia pseudoacacia*, *Rudbeckia hirta*, *Solanum nigrum*, *Trifolium pratense*, and *Verbascum thapsus*. Of the 20 other species that showed some life after 39 years, 18 species had not more than 6 percent of germination at either depth.

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CHECKING OF FRUITS OF THE DEGLET NOOR DATE IN RELATION TO WATER DEFICIT IN THE PALM¹

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INTRODUCTION

Minute cracks in the surface of fruits of the date (*Phoenix dactylifera* L.) may appear during the summer after a period of rain or

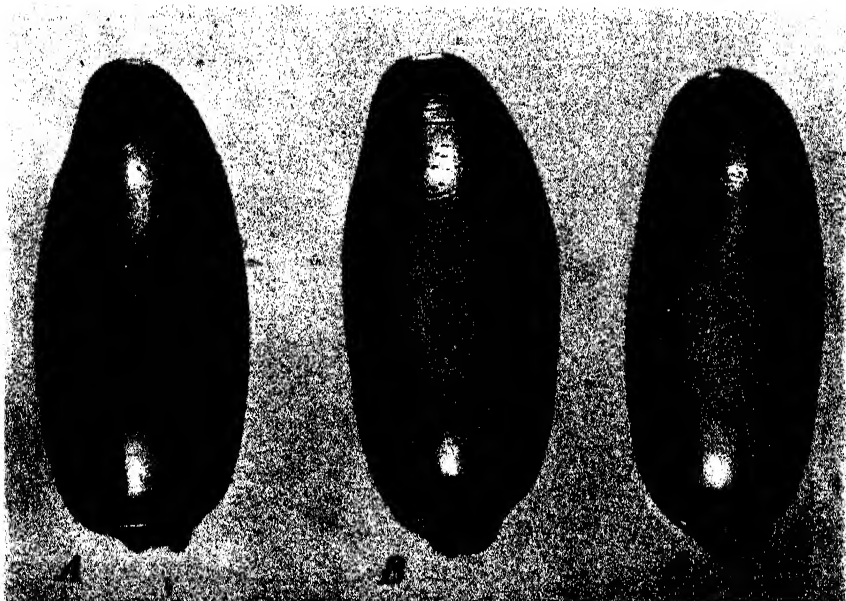


FIGURE 1.—Checking on fruits of the Deglet Noor date on August 10, 1942: A, No visible checking; B, moderate amount of checking; C, severe checking, typical of that which develops into blacknose. The checks on fruit C and on the left edge of fruit B are 4 to 5 days old; the checks in the middle of fruit B are only a few hours old.

high relative humidity (fig. 1, B). Such cracking, known by California date growers as checking, has been observed on fruits of most varieties. On the Deglet Noor variety such checking, if severe

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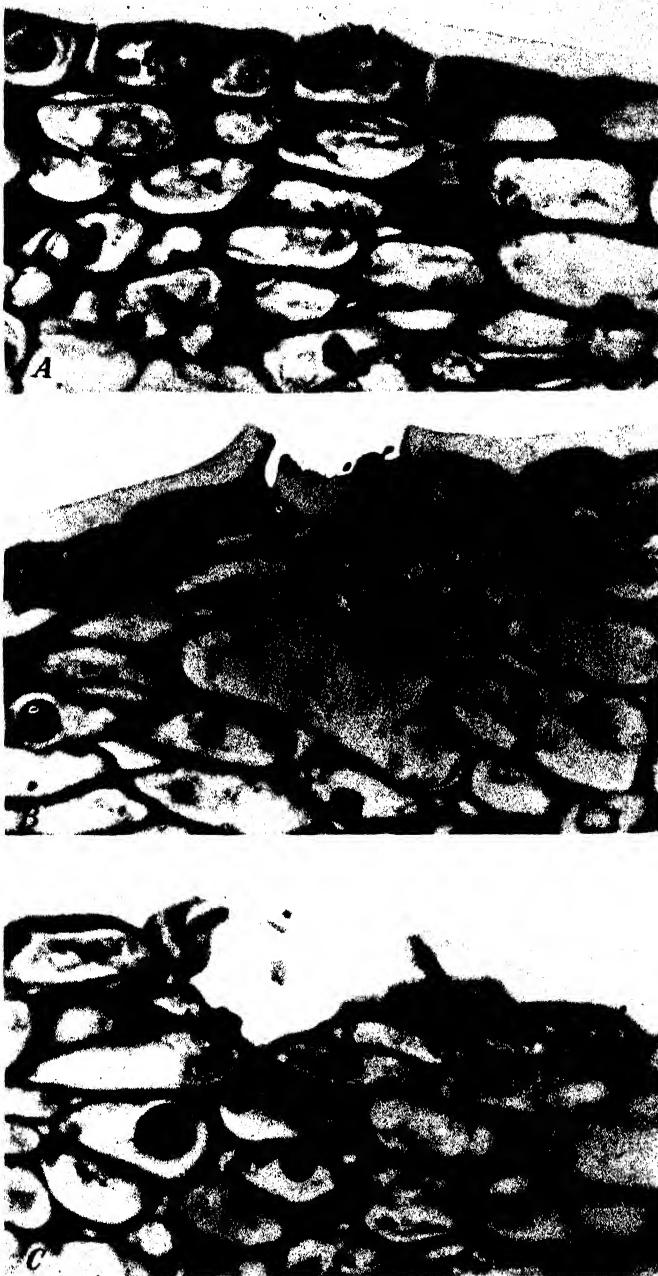


FIGURE 2.—A-C, Radial longisections through epidermal and hypodermal cells of Deglet Noor fruits approximately 10 hours after minute cracks appeared. Note in *A* the minute crack in the cutinous layer, extending into an epidermal cell; in *B* the ruptured epidermal cell, with the extended hypodermal cells just below; and in *C* ruptured cells in hypodermal as well as in epidermal layer. (Photomicrographs by E. M. Long.)

(fig. 1, C), may eventually cause a darkening and shriveling called blacknose. The minute cracks constituting the checking start in the epidermal cells and involve initially only two or three layers of hypodermal cells (fig. 2). Severe checking and blacknose of Deglet Noor appreciably reduce the total production of marketable fruit in the Coachella Valley of California in about 1 out of every 5 years, and in some locations they lower the commercial grade of part of the crop almost every year.

Susceptibility of Deglet Noor fruits to checking ceases when the fruits reach full size and the color changes from yellowish green to pink. Thereafter, while the fruits are pink (the khalal stage), rain injury is manifested either by a splitting, called tearing, or by many long but narrow cracks, which will be referred to as khalal cracking.

Pathological studies reported by Klotz (6)³ and Fawcett and Klotz (3) indicated that no micro-organism was responsible for blacknose, but that showers or periods of high humidity might be involved. An increase in checking or blacknose after late-spring or early-summer bagging of branches was observed by Nixon (9). Klotz (6) was able to produce khalal cracking by placing strands of fruits in chambers at 100, 89.9, and 80.5 percent humidity. In a comprehensive study of checking, Haas and Bliss (5) found that covering fruit bunches with paper tubes during the period of fruit susceptibility to checking increased this injury and separating the strands to facilitate air circulation reduced it. Later Nixon and Crawford (10) showed that reducing the number of fruits per bunch, either by removing flowers at time of pollination in March or by removing fruits on June 1 increased both checking and blacknose.

To obtain a better understanding of the action of water in the development of checking on Deglet Noor fruits, studies were conducted at the United States Date Garden, Indio, Calif., from 1938 to 1941, to ascertain: (1) Period of fruit susceptibility to checking as determined by immersion of detached fruits in water; (2) relation of checking to water intake by detached fruits immersed in water and in sucrose solutions; (3) effect upon checking of varying the water supplied to detached strands of fruits; (4) effect upon checking of various water-vapor-pressure deficits of air surrounding fruits on the palm; (5) rate of transpiration of fruits on the palm; and (6) the degree of checking of fruits on the palm in relation to appreciable water deficits in the palm. The results of the studies are reported herein.

RESULTS

PERIOD OF FRUIT SUSCEPTIBILITY TO CHECKING

Since both Nixon (9) and Haas and Bliss (5) were able to cause Deglet Noor fruits to check by immersing them in water for 24 to 48 hours, a modification of this technique was used to determine the period of fruit susceptibility. During the summer fruits were removed weekly from the bunch between 6:30 and 7:30 a. m., and within an hour they were immersed in water at 26° to 30° C. The severed vascular tissues were not sealed. At the end of 4 hours the fruits were removed and wiped dry, and their surfaces were examined for minute cracks.

³ *Italic numbers in parentheses refer to Literature Cited, p. 231.*

Preliminary work in 1938 showed that the fruits were susceptible to checking throughout July and during part of August and that susceptibility decreased at about the time the fruit reached maximum size and the color changed from yellowish green to pink. These results are in agreement with those of Nixon (9) and of Haas and

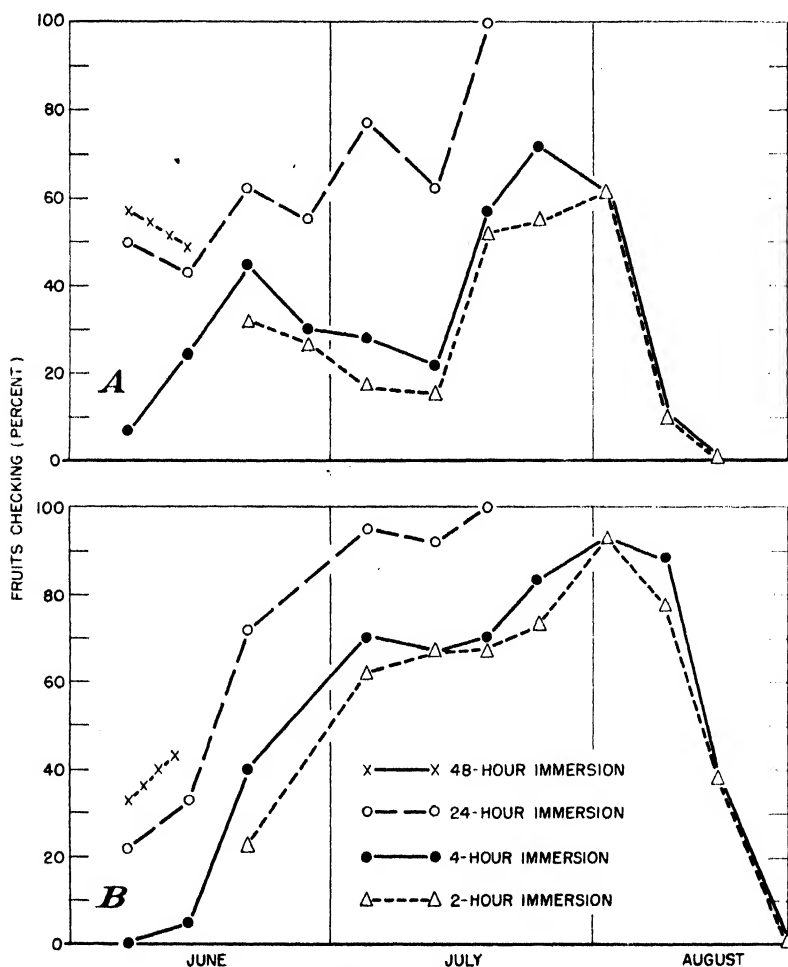


FIGURE 3.—Date fruits checking after different periods of immersion, in weekly samples, 1939: A, From United States Date Garden; B, from Cavanagh garden.

Bliss (5). To determine the time or stage of fruit development at which susceptibility starts and also the effect of the length of the period of immersion of the fruits on checking, fruits (60 in each sample) were collected at weekly intervals in two locations in 1939 and in three locations in 1940. To avoid the inclusion of fruits of widely divergent stages of development, all fruits were obtained from bunches pollinated at approximately the middle of the pollination period. In 1939 the fruits were from bunches pollinated within 7

days of March 19; in 1940, from bunches pollinated within 7 days of March 1.

In 1939 the fruits were examined after 2, 4, 24, and 48 hours of immersion. Since checking after 48 hours of immersion was about the same as that after 24 hours, immersions for 48 hours were discontinued in June. As shown in figure 3, immersion for 2 and 4 hours showed about the same relation between extent of checking and time of collection during the summer. Immersion for 24 hours caused all the fruits to check several weeks before the maximum checking was observed for the 4-hour immersion. The 4-hour period of immersion was therefore selected for determining degree of susceptibility during the summer.

In figure 4 are presented the degree-of-susceptibility curves for fruits during 1939 and 1940 from the control (plot A) of each of several series of irrigation plots in three locations. The dips in the susceptibility curves in July 1939 may have been the result of high maximum temperatures, which at the United States Date Garden ranged from 43° to 48° C. between July 8 and 14. The slightly earlier period of susceptibility to checking there than at the Cavanagh garden is probably related to the earlier fruit development and maturity at the United States Date Garden, which is the warmer location. An abnormally low set of fruit at the Cavanagh garden in 1940 resulted in a much lighter crop than at the other two locations; this lighter crop may have caused the indicated greater susceptibility of the Cavanagh fruit to checking after the 4-hour immersion in water. The dips in the curves for checking in early July 1940 coincide with a period of 13 consecutive days of maximum air temperatures of 43° or more. The time of termination of the period of susceptibility in each of the three locations corresponded to the time at which fruits attained full size and changed in color from yellowish green to pink.

The period of susceptibility to checking in relation to fresh and dry weights of the fruits is illustrated in figure 5. The 10 to 40 percent checking during June occurs at the stage in fruit enlargement when, according to Long (7), cell enlargement in the apical region is nearly complete. The 70 percent or more checking beginning at the end of June coincides with the period when cell enlargement in the median region is about completed. Thus the susceptibility to checking, which is confined to the apical and median regions (fig. 1), may be due to lack of elasticity of walls of cells which have ceased enlargement, or possibly to a reduction in the plasticity of the cuticle overlying the cells that have stopped growing. Maximum susceptibility to checking occurs while the fruits are approaching maximum size, growth being largely the result of enlargement of cells in the basal region (5, 7).

In all five cases where weekly fruit samples were obtained, the relatively abrupt termination of the period of susceptibility to checking was associated with the change from an increase in total amount of water per fruit to a decrease in total amount of water per fruit (fig. 5, C). This would be expected to result in a reduction in the turgor of the fruit as a whole, which would lessen the strain on the walls of the peripheral cells and cuticle, as suggested by Haas and Bliss (5).

When the period of susceptibility to checking ceases, the fruit becomes susceptible to khalal cracking and to tearing. Tearing is

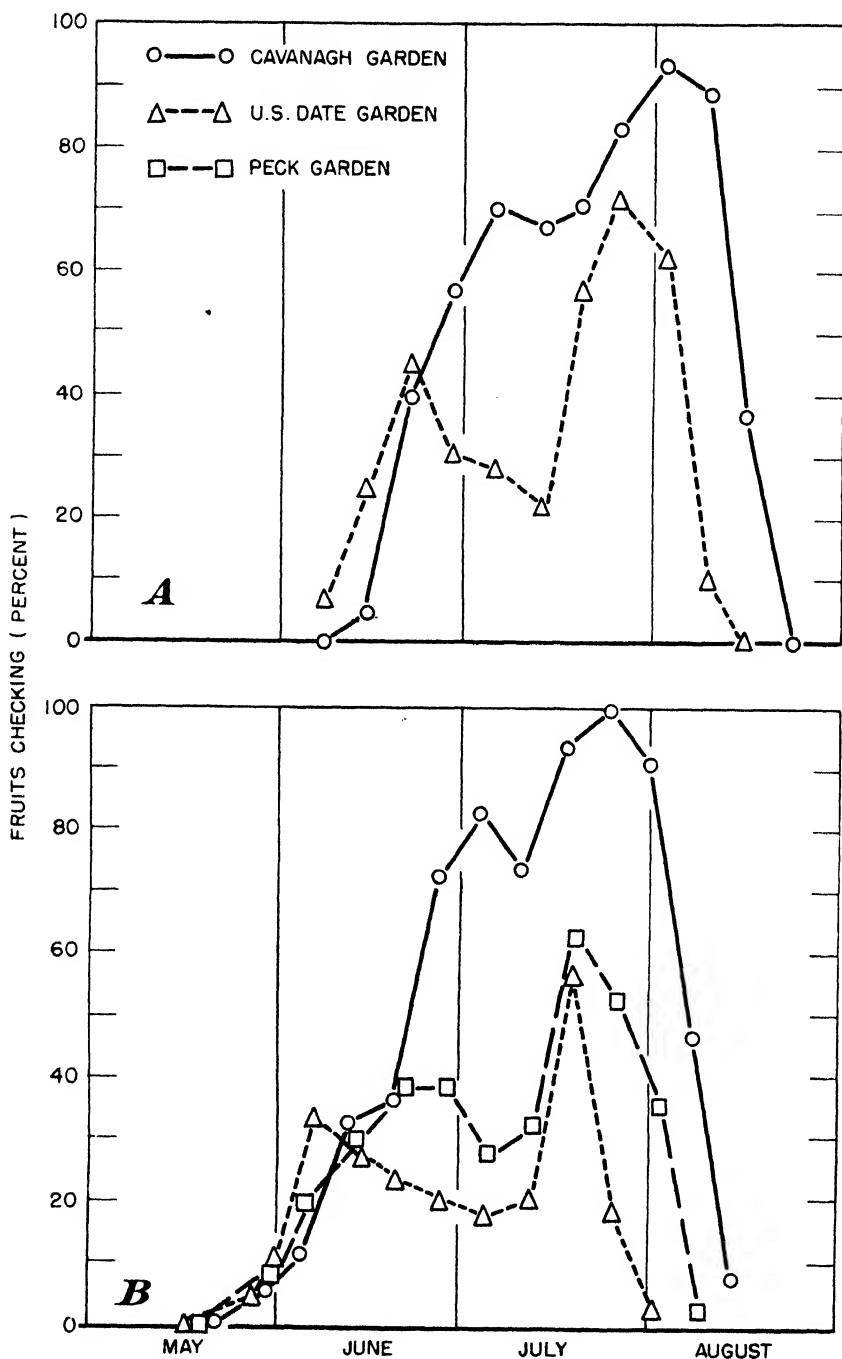


FIGURE 4.—Date fruits checking after a 4-hour immersion in water, in relation to time of collection during the summer: *A*, Cavanagh garden and United States Date Garden, 1939; *B*, Cavanagh and Peck gardens and United States Date Garden, 1940.

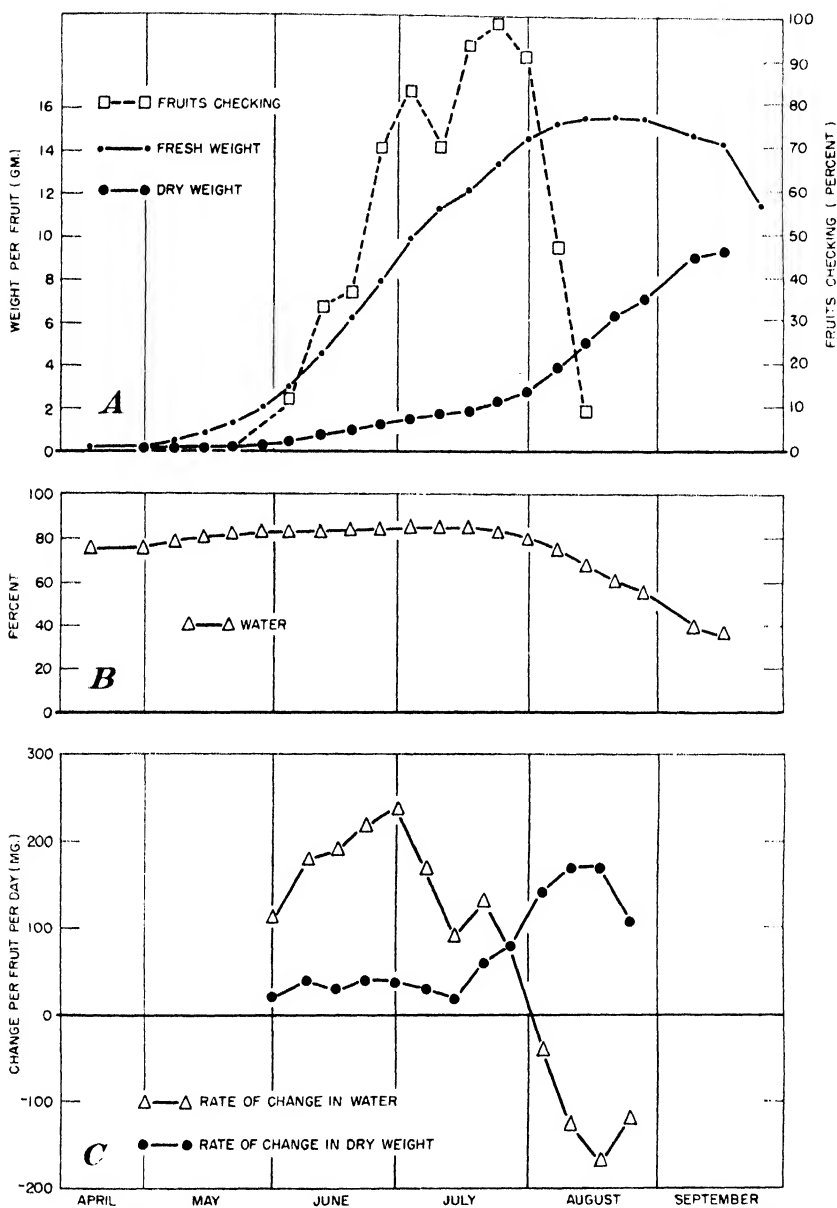


FIGURE 5.—Susceptibility of dates to checking in relation to normal development of the fruits in Cavanagh plot B, 1940: A, Fresh weight per fruit, dry weight per fruit, and percentage of fruits checking; B, percentage of water; C, rate of increase or decrease in water and dry weight per fruit.

the formation of a few long, deep cracks involving mesocarp as well as epidermal and hypodermal cells. In the severest form of tearing the outer mesocarp separates from the inner mesocarp and rolls back.

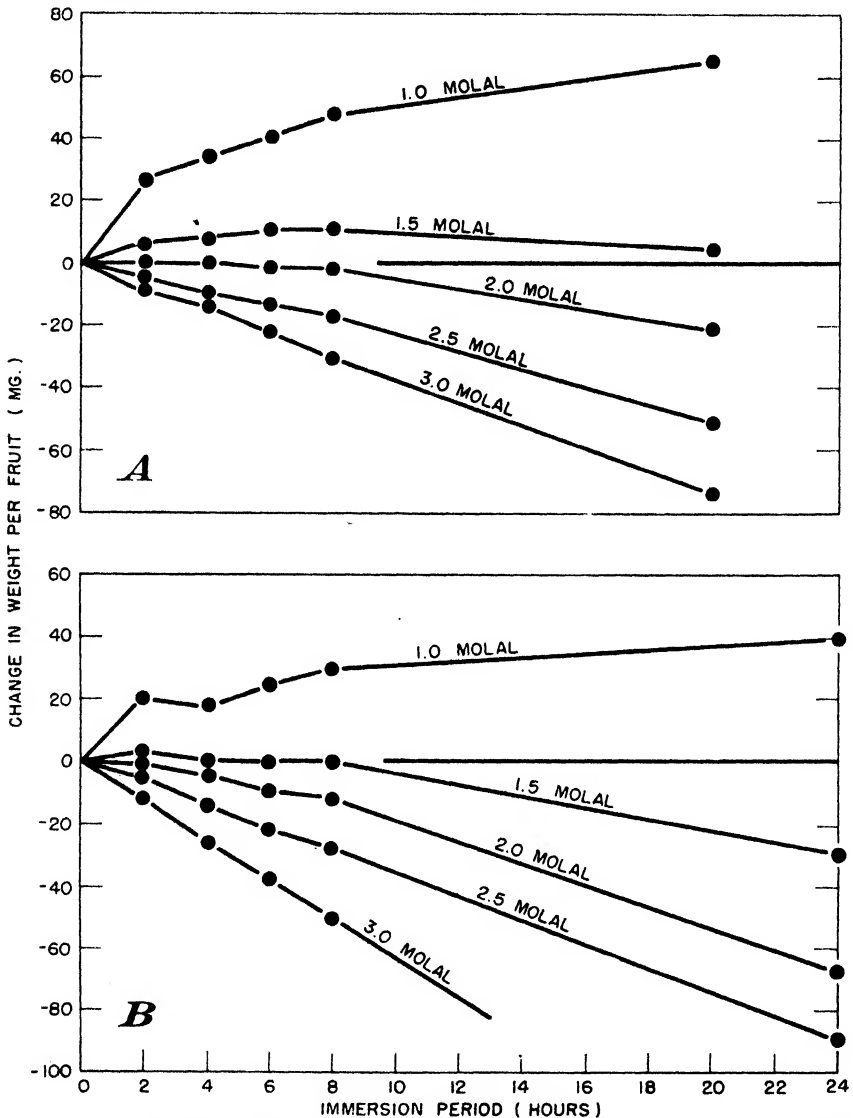


FIGURE 6.—Change in weight per date fruit during different periods of immersion in sucrose solutions of various molal concentrations: A, Fruits immersed at 11 a. m. on June 15; B, fruits immersed at 7 a. m. on June 16.

This change in manner of rupture of the fruit may be related to other developmental changes in the fruit. The rapid increase in dry weight per fruit, shown by Haas and Bliss (5) and by Rygg⁴ to be

⁴ RYGG, G. L. COMPOSITIONAL CHANGES IN THE DATE FRUIT DURING GROWTH AND RIPENING. U. S. Tech. Bul. 910. illus. 1946.

almost entirely due to sucrose accumulation, has begun. This may result in a large increase of osmotic pressure of the cell sap in the mesocarp region of the fruit, which will increase the potential turgor of the cells if their walls are no longer capable of appreciable stretching. Since at this time the inner mesocarp cells have attained their maximum length, according to Long (7), it is likely that very little stretching of cell walls can occur. The susceptibility to tearing ceases when the fruit tissue begins to soften, at which stage the cells apparently lose their semipermeability.

RELATION OF CHECKING TO WATER INTAKE OF DETACHED FRUITS IMMERSSED IN WATER AND IN SUCROSE SOLUTIONS

In a preliminary estimation of the diffusion-pressure deficits in the fruits in 1938, fruits collected on June 13 were immersed in sucrose solutions of 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 molal concentrations. At the end of 2 hours fruits in all solutions had increased in weight about 0.07 gm. per fruit. On June 15 fruits were immersed at 11 a. m. and on June 16 at 7 a. m. in sucrose solutions of 1.0, 1.5, 2.0, 2.5, and 3.0 molal concentrations and then weighed at intervals for 24 hours. As shown in figure 6, fruits in 1.0 molal concentration increased in weight, while those in 2.5 and 3.0 molal concentrations decreased in weight.

On the basis of these results, lots of four fruits were collected weekly before 7:30 a. m. and immersed 4 hours in sucrose solutions of 1.1, 1.3, 1.5, 1.7, and 1.9 molal concentrations, as well as in water. The numbers of fruits developing checking are given in table 1, and the average changes in weight are shown in figure 7.

Although fruits immersed in 1.5, 1.7, and 1.9 molal sucrose solutions consistently decreased in weight, they developed checking nearly as readily as fruits which increased in weight in water. Thus checking was not necessarily accompanied by increased absorption of water by the fruit as a whole, although it probably resulted from water movement into certain cells to the extent that the increased turgor caused a rupture in the cutinous layer and in the epidermal and hypodermal cells. The relatively slight changes in weight of fruits immersed in 1.1 molal sucrose solution indicate that fruit collected in the early morning during July and August developed as a whole a diffusion-pressure deficit of about 30 atmospheres.

TABLE 1.—*Effect of concentration of sucrose solution upon number of fruits showing checking after immersion for 4 hours*

[4 fruits in each lot]

Type of solution	Fruits developing checking, for each collection date											
	July				August				September			
	7	14	21	28	4	11	18	25	8	15	22	
	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	
Water	4	4	4	4	4	4	3	2	2			(1)
1.1 molal	0	4	4	1	4	4	4	2	0	0		(1)
1.3 molal	0	3	4	3	4	3	3	0	0	0		0
1.5 molal	1	4	4	3	4	4	3	2	0	0		0
1.7 molal	3	4	4	4	4	4	3	0	0	0		0
1.9 molal	0	4	4	4	4	4	3	0	0	0		0

¹ Fruits developed tearing.

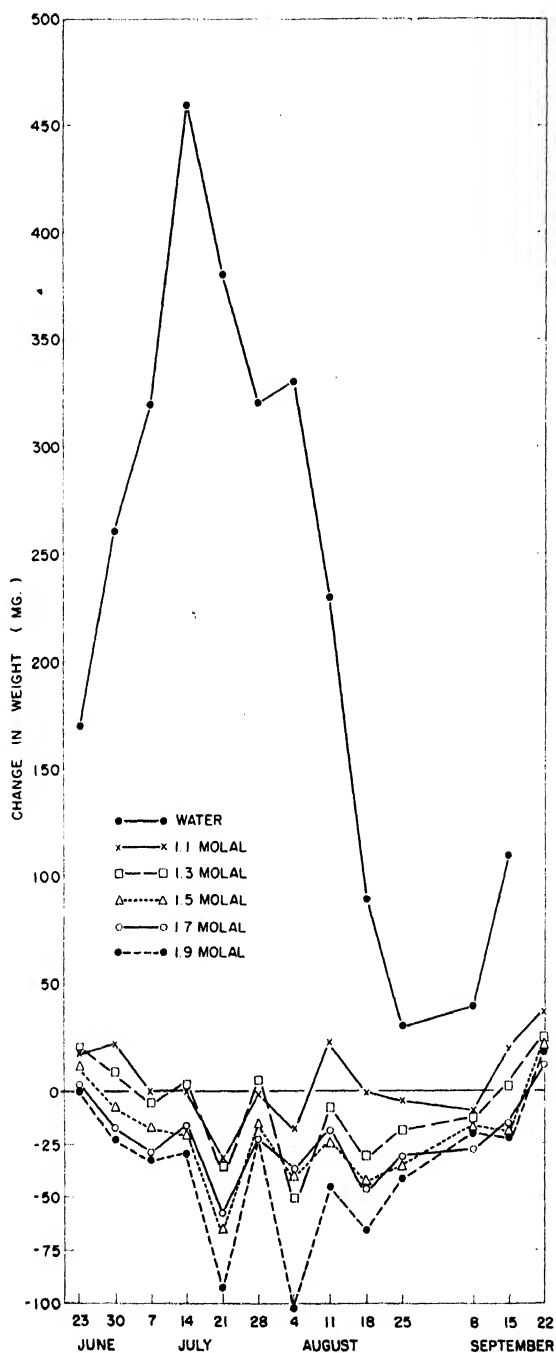


FIGURE 7.—Change in weight per date fruit after 4-hour immersion in water or in sucrose solutions of various molal concentrations.

For fruits immersed in water the trend for increasing amount of water absorption from June 23 to July 14, but decreasing amount of water absorption after July 14, is probably related to the relative proportion of the fruit in which cells were enlarging rapidly. The June 23 to July 24 period coincided with the period of most rapid fruit enlargement, during which Long (7) observed cell enlargement throughout the fruit. After July 4, when fruit enlargement became progressively less rapid, cell enlargement was found to be confined to the basal part of the fruit. Thus it seems likely that the hydration capacity of actively dividing or enlarging mesophyll cells is greater than that of fully enlarged cells.

Morning and afternoon collections of fruits on August 23 were immersed in water and in sucrose solutions (table 2). After immersion the fruits in the morning collection showed some checking, but those in the afternoon collection showed little or none. Since the fruits normally shrink appreciably during the day (8), they presumably have a lower water content in the afternoon, so that a smaller water supply within the fruits in the afternoon than in the morning may have prevented checking. This evidence is interpreted as substantiating a concept that water within the fruit moves into the peripheral cells which develop the excessive turgor that causes checking, while the effect of the solution surrounding the immersed fruit is largely to reduce the rate of diffusion of water out of the fruit and to allow accumulation of water in the peripheral region.

TABLE 2. — *Effect of time of collection upon number of fruits showing checking after immersion in water or sucrose solutions*

Immersion period and collection time	Fruits that developed checking in indicated solution					
	Water	2.0 molal	3.0 molal	4.0 molal	5.0 molal	6.0 molal
	Number	Number	Number	Number	Number	Number
4-hour immersion:						
7:50 to 8:15 a. m.	3	3	3	3	1	1
2:40 to 2:50 p. m.	0	0	0	0	0	0
24-hour immersion:						
7:50 to 8:15 a. m.	4	3	4	3	2	2
2:40 to 2:50 p. m.	1	1	0	1	0	0

A consideration of the probable magnitude of the vapor-pressure gradient of water from epidermal cells of the fruit to the outside air gives plausibility to this explanation of checking. If the concentration of sap in epidermal cells at zero turgor is assumed to be 2 molal, the temperature of fruit and air to be 25° C., and the relative humidity of outside air to be 50 percent, then the water-vapor-pressure gradient from the cells through the cuticle to the air might amount to about 10.89 mm. Hg (22.77 mm. for vapor pressure of cell sap minus 11.88 mm. for vapor pressure of outside air). With such a vapor-pressure gradient causing water to move from the epidermal cells to the outside air, the water vapor might be expected to move through the relatively impervious cuticle at a rate greater than that at which water might be moving from the interior of the fruit to the epidermal cells, and the cells would not develop an excessive turgor. However, when the fruit is immersed in a 3.0 molal sucrose solution, the water-vapor-pressure gradient from epidermal cells to the outside

solution might initially be about 0.59 mm. Hg (22.77 mm. minus 22.18 mm.). Then the rate of water movement from the epidermal cells to the outside solution might become slower than the rate of movement from the interior of the fruit to such cells, resulting in excessive turgor and checking of the epidermal cells.

Additional evidence that water within the fruit may cause the checking was obtained by a simple experiment. Three lots of 20 fruits each were collected in early morning, and the perianths of all fruits were sealed with melted paraffin. One lot was dipped in melted paraffin to seal the entire fruit surface and then was exposed to laboratory air; the second lot was immersed in water; and the third lot was left with the fruit surface exposed to laboratory air. At the end of 4 hours all fruits completely covered with paraffin were checked as much as the fruits immersed in water, but the fruits with their normal surface exposed to air showed no checking. Thus, preventing water movement out of the turgid fruits without allowing water movement into the fruits resulted in checking.

EFFECT UPON CHECKING OF VARYING THE WATER SUPPLY TO DETACHED STRANDS OF FRUIT

To investigate further the relation to checking of water supplied to the fruits through the vascular system and of water vapor in the air surrounding the fruits, the basal end of a detached strand of fruit was connected to a water supply from a burette while another detached strand was left without this supply of water. The two strands were then suspended with the fruits of both enclosed in the same chamber for 6 to 8 hours. Two such chambers were used in each of two experiments. In the first experiment the air in chamber 1 was maintained at about 76 percent relative humidity by passing into it air which had bubbled through a series of saturated solutions of sodium chloride; the air in chamber 2 was maintained at 100 percent relative humidity by warm water in the bottom of the chamber. The second experiment was similar, except that in chamber 1 the air was maintained at about 95 percent relative humidity by passing the air through a series of saturated solutions of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. The air temperatures were about 25° C. The percentages of fruits developing checking are given in table 3.

TABLE 3.—*Effects of water supply to a strand of detached fruit and of relative humidity of the surrounding air upon the percentage of checking, 1938*

Date and relative humidity of air surrounding fruits	Strand with or without water supply	Fruits per strand	Fruits checking
		<i>Number</i>	<i>Percent</i>
July 17:			
76 percent	With	10	60
Do	Without	9	0
100 percent	With	24	100
Do	Without	24	46
July 18:			
95 percent	With	10	100
Do	Without	11	0
100 percent	With	23	100
Do	Without	24	21

The fact that where water was supplied to the strand checking occurred while the fruits were in air at 76 percent relative humidity indicates that the excessive turgor of the peripheral cells can develop

with the vapor pressure of the water in the air surrounding the fruits less than the lowest possible vapor pressure of water in the cell sap, provided the supply of water from the strand to the fruits is relatively great. The greater checking shown by fruits on strands supplied with water than by those on strands not supplied with water, with the same relative humidity of the surrounding air, shows that the availability of water to the fruits from the strand is an important factor in causing checking.

EFFECT UPON CHECKING OF VAPOR-PRESSURE DEFICIT OF AIR SURROUNDING FRUITS ON THE PALM

Although all fruits on detached strands supplied with water developed checking when exposed to air at 95 percent relative humidity,



FIGURE 8. - Apparatus for passing air of approximately constant water-vapor-pressure deficit over date fruits on the palm.

additional work was necessary to determine whether fruits on the palm would be equally susceptible to checking. This determination necessitated a technique that would maintain approximately constant relative humidity of air under field conditions, despite the water from fruit transpiration and the fluctuations in outside air temperatures. The apparatus used is shown in figure 8.

Air was forced by a pump capable of moving 4.3 cubic feet of air per minute against a pressure of 10 pounds through two parallel lines. In one line the air was saturated with water by bubbling it through warm water; in the other line the air was dried by passing it through sulfuric acid. These two air streams were then mixed, and by regulating the relative volumes of air through the two lines the water-vapor

content of the ultimate mixture was adjusted. This final air stream was passed into a chamber enclosing one strand of fruit and was allowed to escape through a tube in the cover. As the escaping air stream had a velocity exceeding 10 feet per second, a wet-bulb thermometer suspended in this escaping air stream gave reliable values. Since the pressure drop from the inside of the chamber to the outer end of the tube in the cover was very small, the water-vapor-pressure deficit of the escaping air stream was probably within ± 0.2 mm. Hg of that of the air around the fruits. Fruit temperatures were assumed to approximate closely those of the dry-bulb thermometer in the air stream. In spite of frequent adjustments of the wet- and dry-air streams, sudden changes of 2° to 3° C. in outside air temperature occasionally caused temporary fluctuations of 1 to 1.5 mm. in vapor-pressure deficit of the air surrounding the fruits. Generally, however, wet- and dry-bulb thermometer readings were kept within $\pm 0.3^{\circ}$ of the desired temperature difference so that vapor-pressure deficit of the air stream was maintained within about ± 0.6 mm. Hg of the desired value. No checking developed on control fruits outside of the apparatus during any of the runs. The percentages of fruits that developed checking in the controlled air stream are given in table 4.

TABLE 4.—*Effect upon the percentage of fruits checking of water-vapor-pressure deficit of air around fruits on the palm*

Run No.	Date	Clock time	Approximate vapor-pressure deficit	Approximate relative humidity	Fruits exposed	Fruits checking
	1939		Mm. Hg	Percent	Number	Percent
1-A	July 27-28	7 p. m.-10 p. m.	1.4	96-95	22	32
1-B		3 a. m.-6 a. m.	1.4	95-94	42	40
2-A		3 p. m.-6 p. m.	1.4	96-95	55	0
2-B	Aug. 1-2	7 p. m.-10 p. m.	1.4	96-95	53	0
2-C		3 a. m.-6 a. m.	1.4	95-94	59	0
2-D		7 a. m.-9 a. m.	1.4	95-94	25	100
3-A	Aug. 2-3	10 p. m.-10 a. m.	1.4	96-94	28	32
3-B		7 a. m.-10 a. m.	1.4	95-94	25	88
4-A		7 a. m.-10 a. m.	2.8	92-91	29	24
4-B	Aug. 3-4	4 a. m.-10 a. m.	2.8	92-91	55	42
5-A		6 p. m.-6 a. m.	2.8	92-91	19	0
5-B		12 p. m.-6 a. m.	2.8	92-91	26	0
6-A	Aug. 9-10	6 p. m.-6 a. m.	1.2	96-95	24	0
6-B		12 p. m.-6 a. m.	1.2	96-95	27	0
7-A		6 p. m.-6 a. m.	.6	98	28	36
7-B	Aug. 10-11	12 p. m.-6 a. m.	.6	98	28	18
	1940					
8-A	July 12-13	6 p. m.-6 a. m.	1.0	97-96	50	84
8-B					10	90
9-A	July 15-16	do	0.5-1.0	98-96	54	96
9-B					10	100
10-A	July 16-17	do	3.0	91-90	53	52
10-B					11	100
11-A	July 23-24	do	3.0	91-90	54	93
11-B					9	100

¹ Strand severed from bunch, but fruits were inside the chamber as in other runs. The cut end of the strand was supplied with water under a small force.

² Strand left attached to palm, but fruits were inserted in a thermos jug in still air.

The exposure of fruit on the palm to air with a relative humidity of 91 to 96 percent did not cause any checking during some nights, but during others it resulted in the checking of as many as 93 percent of the fruits. However, when the strands were severed and supplied

with water (runs 2 to 4), the checking of fruits was greater on these severed strands than on those still connected to the palm. This indicates that the water-vapor-pressure deficit of the air was low enough and the period of exposure long enough to cause checking, but the water supply from the palm to the fruit was not adequate. However, when weather conditions with high relative humidity cause checking of fruits, the rate of water movement out of the leaves as well as out of the fruits is reduced to the extent that the turgor of the palm as a whole is increased sufficiently to increase the supply of water from the palm to the fruits. Since the effects of weather conditions upon the turgor of the palm may have persisted for several days, it was not possible from the weather records available to relate the results in this experiment to weather except in the most general terms. Apparently cloudiness and a maximum air temperature under 40° C. during the day preceding the night of the run favored the development of checking, whereas clear skies and temperatures of 42° or more during the day preceding the run were unfavorable for checking.

When fruits on a strand attached to the palm were enclosed during the night in a thermos jug (runs 8 to 11), by morning droplets of water were scattered over the inner surface of the jug and over the surface of the fruits, apparently as a result of transpiration and a decrease in temperature of the air within the jug. The fact that there was more checking of fruits in the jug than of those in the air stream showed that for the same water supply from the palm to the fruits a reduction in water-vapor-pressure deficit of the air surrounding the fruits from about 1 mm. Hg to approximately 0 increased the percentage of fruits developing checking. Thus, these results tend to confirm the hypothesis that any condition that favors increase in turgor of the cells of the peripheral region, either by reducing water loss from the fruits or by increasing translocation of water into the fruits, favors checking.

RATE OF TRANSPIRATION BY FRUITS ON THE PALM

The evidence indicating that checking tended to be increased by a relatively slow rate of water loss from the fruits prompted an attempt to measure the rate of transpiration by fruits on the palm. In 1939 this was estimated by determining the difference in moisture content between a stream of outside air after it had passed over two or more strands of fruits (60 to 70 fruits) in a shaded glass chamber and a similar stream before it passed over them. The air stream of 283 to 340 liters per hour was sufficiently rapid to prevent large changes in temperature or relative humidity of the air while in contact with the fruits. The resistance to flow through the large-diameter, short inlet tube caused very little reduction of pressure in the chamber below that of the outside air.

Each air stream was drawn through flexible copper tubing to a train consisting of (1) two gas washing bottles of concentrated sulfuric acid (in which the water vapor was absorbed and weighed), (2) a flowmeter (used as indicator of the rate of flow), (3) two gas washing bottles of water (to saturate air with water vapor), and (4) a wet gas meter (to measure the total volume of air passing through the train). Pressures in the wet test meters were held practically constant by

slight adjustments of needle valves between the meters and the vacuum pump. Temperatures and pressures in the meters were read at intervals and averaged for a run. The observed volumes of air were corrected to standard conditions, and the average loss of water per fruit per hour was calculated for each run.

The average transpiration rate of fruits on one palm in each of the irrigation plots A (control) and BC (appreciable water deficits in the palm from June 16 to August 17) at the United States Date Garden was determined several times during the summer for 24-hour periods, divided into six runs of about 4 hours each. The average transpiration per fruit per hour is shown in figure 9. The observed transpiration rates of the fruits in plot BC were considerably lower than those in plot A until after August 17. This indicates that while there were appreciable water deficits in the palm in plot BC, the rate of transpiration of the fruit was reduced by a limitation in the supply of water from the palm to the fruits. During the daytime in July and August the indicated average rate of transpiration was about 40 mg. of water per fruit per hour and was never found to exceed 60 mg. These values are only slightly higher than those estimated by Haas and Bliss (5), based upon repeated weighings of detached, nearly ripe fruits on September 13, 1933.

The highest rate of transpiration in the 24-hour period was, as might be expected, usually during the 4-hour period at midday or during the early afternoon. From this maximum, transpiration decreased during the afternoon and night to a minimum during the early morning, at which time air temperature and vapor-pressure deficit were also at a minimum. After the relatively humid night of August 2-3, when the average rate of transpiration became as low as 6 mg. per fruit per hour, several large fruits on heavily thinned bunches were observed to develop fresh checks at sunrise.

The capacity of transpiration by the fruits to increase the water vapor in the air surrounding them may be one of the factors that cause the checking. A bunch of 1,000 fruits, each transpiring at the rate of only 10 mg. per fruit per hour, could saturate 100 liters of still, dry air (at 25° C.) in 18 minutes. This emphasizes the importance of the results of Haas and Bliss (5) showing that conditions which reduce the circulation of the air in the fruit bunch increase checking. However, when weather conditions reduce the vapor-pressure deficit of the air, transpiration by the leaves as well as by the fruits is retarded. As pointed out earlier, a retardation of transpiration by the leaves would be expected to favor checking of fruits by increasing the supply of water from the palm to the fruit.

REDUCTION OF CHECKING OF FRUITS ON THE PALM IN RELATION TO APPRECIABLE WATER DEFICITS IN THE PALM

The evidence thus far points to water deficits in the fruit resulting either from a greater rate of water movement out through the fruit surface than into the fruit from other parts of the palm or from withdrawal of water from fruit by transpiring leaves as factors reducing fruit susceptibility to checking. Since appreciable water deficit in the palm resulting from soil-moisture deficiency should materially reduce water movement from palm to fruit and thereby increase water deficit in the fruit, such water deficit in the palm should reduce

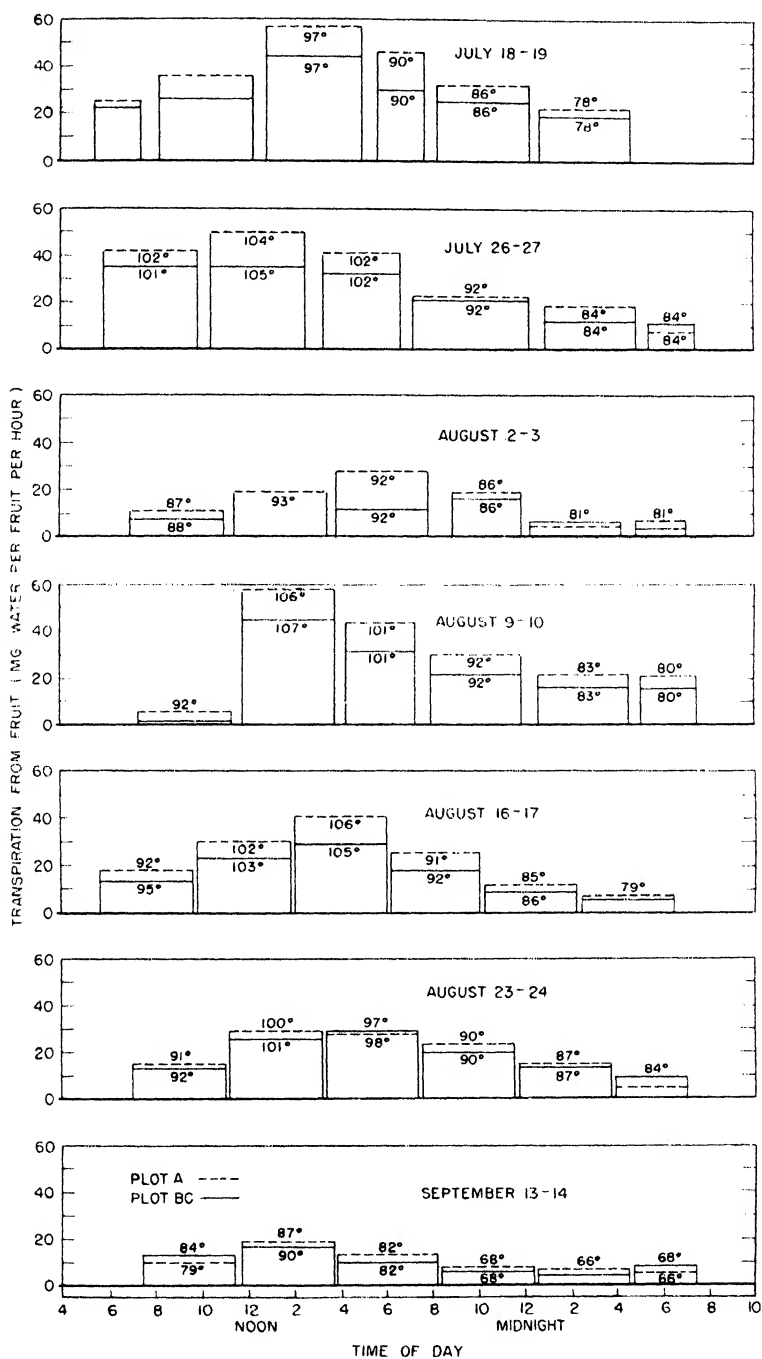


FIGURE 9.—Average rate of transpiration of date fruit for 4-hour periods in seven runs, for one strand of fruit in irrigation plot A and plot BC, respectively, at the United States Date Garden, 1939. The average temperature (° F.) of the air surrounding the fruit in each chamber is indicated for each run.

checking. Several series of irrigation plots, described elsewhere (1, 8), provided an opportunity to relate the percentage of fruits developing checking or blacknose to appreciable water deficit in the palm resulting from soil-moisture deficiency. With the omission of irrigations in a certain plot, appreciable water deficits in the palms in that plot were indicated when the rate of leaf elongation became 0.5 cm. or more per day lower than that of regularly irrigated palms (representing the commercial practice or "control"). After the resumption of irrigation of palms that had been showing appreciable water deficits, the rate of leaf elongation did not immediately increase to that of the control palms; but for this discussion the period of appreciable water deficits will be considered as terminated when the period of soil-moisture deficiency ended. The average percentages of fruits in each plot showing checking or blacknose (table 5) were based upon counts of fruits on two inside and two outside strands on each of three bunches per palm pollinated at the peak of inflorescence emergence.

TABLE 5.—*Effects of appreciable water deficit in palms, as measured by reduced rate of leaf elongation below that in control plot, upon percentage of fruits showing checking or blacknose*

Year and location	Grower's practice or control ¹		Plots showing appreciable water deficit during indicated periods ¹				
	Plot	Fruits showing		Plot	Period of water deficit	Fruits showing	
		Check- ing	Black- nose			Check- ing	Black- nose
1938:		<i>Percent</i>	<i>Percent</i>			<i>Percent</i>	<i>Percent</i>
Martinez Indian Reservation	C		16	A	July 10-Aug. 7		1
				B	July 30-Sept. 8		2
1939:							
United States Date Garden	A	38	0	BC	July 16-Aug. 17	9	0
				B	June 18-July 18	20	1
Cavanagh garden	A	33	3	C	July 4-Aug. 4	22	1
				D	Aug. 3-Aug. 30	31	3
1940:							
Cavanagh garden	A	69	30	B	June 13-July 4	34	2
				C	July 10-Aug. 7	1	0
				D	Aug. 20-Sept. 17	55	15
Peck garden	A	39	12	C	July 10-Aug. 4	6	1
	A-1	15	10	C-1	July 1-Aug. 6	2	1
Martinez Indian Reservation	A-2	12	3	C-2	July 1-Aug. 6	0	0
	A-3	9	3	C-3	July 1-Aug. 6	0	0

¹ Plot designations are those used in other publications (1, 8) referring to these irrigation plots.

From table 5 it is evident that in all 10 plots where omission of irrigation caused appreciable water deficits in the palm at any time from the middle of June to the early part of August the percentage of fruits that developed checking was smaller than in comparable control plots. That period has already been shown (fig. 4) to correspond in 1939 and 1940 to the developmental stages of the fruit when it is susceptible to checking. In the location used in 1938 the fruit remained susceptible to checking until after August 18. Therefore it is not surprising that the appreciable water deficits from July 30 to September 8, 1938, also reduced the percentage of blacknose. On the other hand, at the Cavanagh garden in 1939 and 1940 the periods of water deficits that started in August, just before the termination of

the period of susceptibility to checking, appeared to have little or no effect upon the percentage of fruits developing checking.

DISCUSSION

Frazier (4) and Brown and Price (2) found in the relatively humid Eastern States that irrigation increased the cracking of tomato fruits. Presumably the unirrigated plants developed appreciable water deficits during periods without rain. Werner and Dutt (14) reduced cracking of Triumph potato tubers by root cutting, which apparently reduced water supply from plant to tuber.

The data do not show any indication that irrigation following a retardation in fruit growth during the period of normal fruit susceptibility to checking increased the tendency to checking, as observed by Frazier (4) in the cracking of tomatoes. On the contrary, appreciable water deficits during the first half of the period of normal fruit susceptibility to checking in the date (plot B at Cavanagh garden in 1939 and 1940), followed by regular irrigation during the latter half of the susceptibility period, resulted in a smaller percentage of fruits checking than in the control. Weekly sampling of fruits showed that the water deficit in plot B, as compared with plot A (control), reduced the size of the fruit and the rate of dry-matter accumulation in the fruit during late August or during September (1). Consequently, the water deficit in the palm in late June and early July may have had some persisting effect upon the fruit which caused it to be less susceptible to checking after the regular 10-day irrigations had been resumed.

The data showing less checking in detached strands of fruits without water than in those supplied with water suggest that the appreciable water deficits in the palm resulting from soil-moisture deficiency prevent checking by reducing the rate of water movement from the palm to the fruits. The association of reduced checking with the slightly lower rate of transpiration in the comparison of plot BC with plot A (table 5 and fig. 9) is supporting evidence. Other evidence, however, indicates that one or more factors other than reduced rate of water movement to the fruits are also operative. Appreciable water deficits in the palm as a result of soil-moisture deficiency prior to the middle of July were effective in reducing checking several weeks later, when a resumption of irrigation had presumably provided a normal supply of water from the palm to the fruits. Since these water deficits also reduced the size of the fruits and retarded the rate of dry-matter accumulation in the fruits during the late summer (1), it might be supposed that the water deficits effective in reducing checking had a profound influence upon the physical and chemical condition of the critical peripheral cells.

Long (7) observed that cell enlargement in the median regions of the date fruit continued into July. Therefore, in the case of appreciable water deficit before the middle of July, the cells in the distal part of the fruits may have remained smaller than those of fruits on palms without such water deficit. With their reduced wall surface, such smaller cells would, if wall thickness were not affected, be expected to resist greater turgor pressures than larger cells.

Since the period of fruit susceptibility to checking coincides with the latter part of the period of fruit enlargement, the tendency of the walls of the epidermal and hypodermal cells to rupture may be

directly related to a strain on the walls while final fruit enlargement occurs, as Verner (12, 13) suggested in the case of Stayman Winesap apples. The extension of peripheral cells during the longitudinal growth of the date fruit, which has been observed by Long (7), is shown in figure 2. By reducing the growth of cells causing fruit enlargement, appreciable water deficits may reduce the forces which, by stretching the walls of the critical epidermal and hypodermal cells, make the cells more subject to rupture during abnormally high turgor. That susceptibility to checking is closely related to factors affecting date-fruit growth has already been pointed out by Nixon and Crawford (10, 11), who found that fruit thinning that increased fruit size also increased checking and blacknose.

SUMMARY

To learn more of the role of water in the checking (a type of cracking) of date fruits, the amount of checking of both detached fruits and fruits on the palm was studied in relation to the internal water supply of the fruit and to the water in the medium surrounding the fruit.

Fruits detached from the palm in the morning developed checking after immersion in water for 4 hours. Repetition of this test at weekly intervals during the summer showed that the period of fruit susceptibility to checking began in June and ended in August when the fruit color had changed from yellowish green to pink. The relatively abrupt termination of the period of susceptibility to checking coincided with the developmental stage at which the total weight of water per fruit stopped increasing and began to decrease. When fruits detached in the morning were immersed in 1.5, 1.7, and 1.9 molal sucrose solutions, the fruits decreased in weight but developed checking nearly as readily as fruits immersed in water. The suggested explanation is that during immersion the rate of water movement from the peripheral cells through the cuticle to the sucrose solution was slower than the rate of water movement from the interior of the fruit to these peripheral cells, resulting in excessive turgor and rupture of the cells. Fruits collected in the morning and covered with paraffin checked as much in air as comparable fruits immersed in water, showing definitely that checking can occur without the entrance of water through the surface of the fruit.

When fruits on detached strands were exposed to air at relative humidities of 76, 95, and 100 percent, respectively, the checking was greater if water was supplied to the cut end of the strand. This indicated the importance of water movement to the fruit through its vascular system as a cause of checking. When the exposure of fruits on the palm to air of a relative humidity of 91 to 96 percent did not result in as much checking as when the strand was severed and supplied with water, the checking of the fruit on the palm was apparently being limited by insufficient translocation of water from the palm to the fruit.

The rate of transpiration of fruit during July and August was found to vary from a maximum of 30 to 60 mg. of water per fruit per hour at midday or early afternoon to a minimum of 5 to 20 mg. in the early morning. A generally lower rate of transpiration by fruit on a palm with soil-moisture deficiency than by fruit on a palm with adequate soil moisture indicated that with appreciable water deficits in the

palm the rate of movement of water from the palm to the fruit was reduced.

In a series of irrigation plots, an increased water deficit in the palm as a result of soil-moisture deficiency, in comparison with slight water deficits in adequately irrigated palms, invariably reduced the percentage of fruits that developed checking, provided the increase in water deficit occurred during the period of fruit susceptibility to checking. This reduction in susceptibility of fruit to checking caused by appreciable water deficits may have resulted from a reduction in the supply of water from the palm to the fruit, a reduction in the size of cells, or a reduction in the amount of stretching of the walls of peripheral cells.

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SPORIDIAL FUSION IN *USTILAGO MAYDIS*¹

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INTRODUCTION

Cytological and cultural investigations of *Ustilago maydis* (DC.) Cda.³ reported in the literature have left certain phases of the knowledge concerning sporidial fusion and nuclear phenomena in this species in a state of controversy. Maire (10)⁴ in 1898 suggested that fusion of sporidia occurred in culture. He showed one illustration of fusion but did not describe the nuclear condition. Later Sartoris (14) reported the occurrence of sporidial fusion in culture but did not describe or illustrate the process. Seyfert (15) was unable to confirm the work of Sartoris. Rawitscher (11) reported that the sporidia neither fused nor formed true mycelium but remained uninucleate. Hanna (4, p. 430) studied the physiology and cytology of sporidia in *U. zeae* and *Sorosporium reilianum* (Kuehn) McAlp. and concluded that "... it seems clear that the conditions which stimulate this process in many other smuts are without effect on the sporidia of *U. zeae*." Christensen (2) found it necessary to inoculate the host plant with paired cultures in order to study segregation of sex factors. Sleumer (16) obtained sporidial fusion in culture between compatible sporidia in *U. zeae*. His descriptions and illustrations of the process agree essentially with those given for other members of the Ustilaginaceae.

In view of the conflicting and incomplete information contained in the literature, the work reported herein was undertaken to determine (1) conditions conducive to sporidial fusion in culture and (2) the nuclear behavior following fusion.

MATERIAL AND METHODS

The chlamydospore material of *Ustilago maydis* used in this investigation was collected from inbred lines of corn (*Zea mays* L.) grown at Madison, Wis. Sporidial cultures of monobasidiospore (monosporidial) origin were used and were established by taking single basidio-

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² Grateful acknowledgment is due Dr. J. G. Dickson for helpful suggestions and criticisms throughout the course of this investigation.

³ Its synonym, *Ustilago zeae* (Beckm.) Unger, is used in many articles cited

⁴ Italic numbers in parentheses refer to Literature Cited, p. 242.

spores from the basidium (promycelium) by the method described by Hanna (3).

Stock cultures were maintained on potato-dextrose agar. In the sporidial studies 1-percent plain, refined agar medium or dilute malt-extract liquid medium was used. A malt-extract stock solution was prepared from freshly ground malt used at the rate of 10 gm. per 100 cc. of distilled water. The extraction was carried out at temperatures of 40°, 50°, and 65° C. for successive periods of 10, 20, and 30 minutes, respectively. The liquid was then decanted and autoclaved. A subsequent filtration to remove the precipitate occasioned by autoclaving was necessary if a clear liquid was desired. This stock solution was used at the rate of 1 part to 100 parts of distilled water, unless otherwise stated.

Cultures of paired monosporidial lines were prepared for study on ordinary glass slides or as hanging-drop cultures on deep-well slides. Sporidia from 3- to 4-day-old potato-dextrose agar slants were used throughout these studies.

RESULTS

PHYSIOLOGY OF SPORIDIAL PRODUCTION AND FUSION

Sporidial fusion in certain of the Ustilaginaceae has been reported to be influenced by the nutrient concentration and reaction of the culture media (5, 6, 14). Sleumer (16) observed that sporidial fusion in *Ustilago zeae* occurred on 3-percent maltose or glucose media only after the nutrients were spent. However, he reported fusion also on malt-extract medium after 24 hours. He believed that fusion was most likely to occur when the reaction of the medium was pH 8.0 to 8.5. Kernkamp (7) found three growth types in *U. zeae*: (1) A sporidial type, (2) a mycelial type, and (3) different intergrades of an intermediate type. The growth type used in the present studies obviously was of the intermediate type in that it produced both sporidia and mycelium. Several preliminary tests were conducted with a mixture of compatible monosporidial cultures that were known to cause gall formation when inoculated into corn plants. These tests indicated that (1) the development of haploid hyphae by the sporidia and (2) the fusion of compatible sporidia occurred only in cultures of low nutritive value. Only vegetative budding of the sporidia was observed in cultures with nutrient concentrations common in many laboratory media. These findings are in agreement with those of Kernkamp (7), who concluded that increasing the quantity of various nutrients, especially certain sugars, increased the sporidial growth in cultures of the intermediate type. He reported also that some cultures of the intermediate type were predominantly sporidial in nature when grown in solutions of relatively high nutritive concentration. However, as the cultures aged and the supply of nutrients decreased, the cultures became more mycelial in nature.

Additional tests were made with the same monobasidiospore lines in hanging-drop cultures in sterile distilled water and in malt extracts of 1 and 2 percent. After sterilization, the reaction of distilled water ranged from pH 7.0 to 7.2 and that of the malt extracts from pH 6.8 to 7.0. These cultures were incubated at various temperatures, of 4° intervals, from 4° to 36° C. and were examined after 24, 48, and 72 hours. Each test was made in duplicate and repeated once. The average results of the tests are given in table 1.

At the end of 24 hours, haploid hyphae and fused sporidia were evident in the distilled-water cultures incubated at temperatures of 20° C. or above. Sporidial production and growth rather than fusion appeared to be stimulated slightly in the 1-percent malt-extract cultures. At the end of 48 hours, in the 2-percent malt-extract cultures, the fewest haploid hyphae occurred and there were no sporidial fusions at any temperature. At the end of 72 hours, the percentages of haploid hyphae and sporidial fusions in the 2-percent malt-extract cultures had increased slightly, except at the lowest temperatures, but the percentages were still the lowest. In the cultures in all three media sporidial fusions were found to increase with advancing age. At two temperatures, approximately 6 percent of the sporidia in the distilled-water cultures had fused at the end of 72 hours.

TABLE 1.—*Influence of time, temperature, and culture media on the development of sporidia, haploid hyphae, and fusion pairs in Ustilago maydis*

Time (hours)	Temperature	Distilled water				1-percent malt extract				2-percent malt extract			
		Sporidia		Haploid hyphae ²	Fusion pairs ²	Sporidia		Haploid hyphae ²	Fusion pairs ²	Sporidia		Haploid hyphae ²	Fusion pairs ²
		Production ¹	Length ¹			Production ^{1,2}	Length ¹			Production ¹	Length ¹		
	° C.			Percent	Percent			Percent	Percent			Percent	Percent
24	36	2	2	25	T	2	2	10	0	3	3	0	0
	32	2	2	25	T	2	2	10-12	T	3	3	0	0
	28	2	2	20	T-1	2	2	10-12	T	3	2	0	0
	24	1	2	15	T-1	2	2	10	T	3	2	0	0
	20	1	1	10	T	2	2	5	0	3	2	0	0
	16	0	1	5	0	1	1	1-2	0	2	2	0	0
	12	0	1	0	0	1	1	0	0	1	1	0	0
	8	0	1	0	0	T	1	0	0	1	1	0	0
	4	0	1	0	0	T	1	0	0	1	1	0	0
	36	2	3	40		3	4	20		4	4	3	0
48	32	2	3	40		3	3	20	2-5	4	4	3	0
	28	2	2	30	3	3	3	20	2-5	4	3	2	0
	24	2	2	25	3-4	2	2	15	2	4	3	1-2	0
	20	1	1	15	3	2	2	10	1	3	2	T	0
	16	1	1	5	T-1	2	2	5	0	3	2	0	0
	12	1	1	0	0	1	1	T	0	2	1	0	0
	8	1	1	0	0	1	1	0	0	1	1	0	0
	4	1	1	0	0	1	1	0	0	1	1	0	0
	36	2	3	40		3	4	30		4	4	5	T
	32	2	3	40		3	3	30	3	4	4	5	T
72	28	2	2	30	4-6	3	3	30	3	4	4	3	T
	24	2	2	30	6	3	2	25	3	4	3	3	1
	20	2	2	25	3-4	2	2	20	1	3	3	1	T
	16	1	1	10	T	2	2	10	T	3	2	T	0
	12	1	1	0	0	1	1	T	0	2	1	0	0
	8	1	1	0	0	1	1	0	0	1	1	0	0
	4	1	1	0	0	1	1	0	0	1	1	0	0
	36	2	3	40		3	4	30		4	4	5	T
	32	2	3	40		3	3	30	3	4	4	5	T
	28	2	2	30	4-6	3	3	30	3	4	4	3	T

¹ 0 = No increase; 4 = maximum increase; based on mean values.

² T = Trace.

The temperatures at which the cultures were incubated also influenced the incidence of sporidial fusion. Sporidia produced at the higher temperatures, 28°, 32°, and 36° C., were long and narrow. This condition, together with the rapid multiplication of sporidia by vegetative budding at these temperatures, made the identification of haploid hyphae and fusion pairs difficult and uncertain. In general, a decrease in temperature was accompanied by decreases in the number and size of sporidia, in the number and length of haploid hyphae,

and in the prevalence of fused sporidia. No sporidial fusion and only a small amount of growth occurred in the cultures held at 12° or lower for 72 hours. These cultures and those incubated at the lower temperatures were continued for an additional 7-day period. At that time a few fused sporidia were observed both in the distilled-water and in the 1-percent malt-extract cultures incubated at 12°. No fusion was observed at the lower temperatures.

For a closer study of sporidial fusion, cultures were prepared from paired sporidial lines of monobasidiospore origin in sterile distilled water and in 1-percent malt-extract solutions. These cultures were incubated at 20° and 24° C. Fusion was observed first after 15 and 20 hours in the malt-extract cultures incubated at 24° and 20°, respectively, and after 20 hours in the distilled-water cultures incubated at 24°. Fusion pairs were not abundant, however, until the cultures were 40 to 48 hours old. This confirms the work of Sleumer (16) with

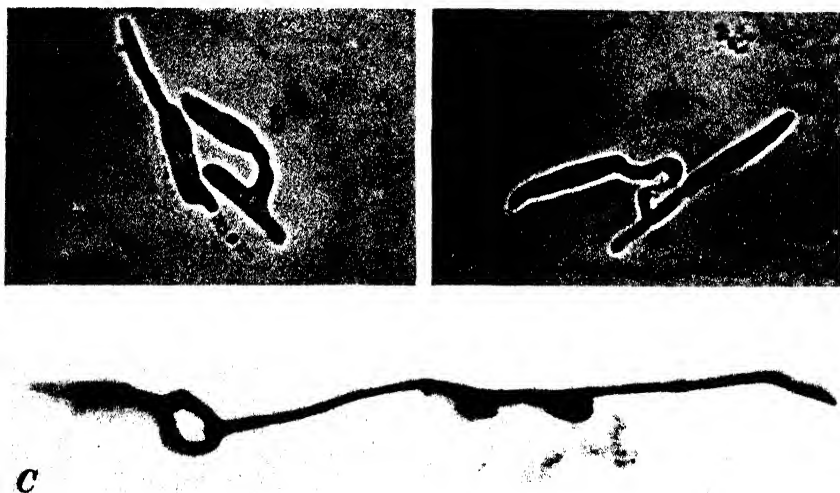


FIGURE 1.—Sporidial fusion in *Ustilago maydis*. Approximately $\times 1,200$. A, *a*, Sporidium germinating, with uninucleate hyphae developing from both ends of the spore; *b*, an early stage of fusion between two compatible sporidia, with the binucleate hypha developing from the end cell of one sporidium. B, An early stage of fusion between sporidia, similar to that in A, *b*. C, A later stage in sporidial fusion, in which the fusion hypha is several cells long. A and B were stained with acid fuchsin and C with Delafield's haematoxylin.

the same fungus in respect to time required for fusion to take place. The fusion process between compatible sporidia of *Ustilago maydis* differs only slightly from that reported for certain other smuts. The conjugation process did not seem to follow any definite pattern such as that described by Holton (6) for *U. avenae* and *U. levis*. Neither sporidium appeared to take a more active part than the other. Fusion appeared to fall into three general types: (1) Direct fusion of two adjacent sporidia; (2) fusion of young one- or two-celled hyphae (germ tubes) formed from sporidia close to each other; and (3) fusion of cells of two older uninucleate mycelia. The uninucleate hyphae by means of which the fusions occurred were found to vary considerably in diameter and length. Representative stages of fusion are

shown in figure 1. In figure 1, *A, a*, uninucleate hyphae have developed from both cells of a two-celled, nonfused sporidium. Early stages of fusion are illustrated in figure 1, *A, b*, and *B*. In each case the fusion hypha has just started its development. A somewhat later stage in which the fusion hypha is several cells in length is shown in figure 1, *C*. In this case the sporidia have not fused directly although in juxtaposition, but rather fusion has taken place between the short hyphae developed from each.

Although fusion was more commonly observed between compatible sporidia close to each other, it was observed also between cells of uninucleate hyphae originating from widely separated sporidia. This type of fusion is not peculiar to *Ustilago maydis*, as a similar type of fusion has been reported by Stakman, Cassell, and Moore (17) for *Urocystis occulta* (Wallr.) Rub. In a few instances compatible sporidia that had apparently fused end to end by means of short germ tubes were observed. In such cases the binucleate hypha developed from the opposite end of one sporidium.

The hyphal outgrowth initiating the binucleate condition develops soon after the fusion of two compatible gametes. The hyphae grow rapidly and under favorable conditions often reach a considerable length. Occasionally it was possible to follow binucleate hyphae that had grown across the culture drop for distances of approximately 5 to 8 mm. (5,000 μ to 8,000 μ). Sleumer (16) observed one such hypha that had reached a length of 340 μ .

Holton (6) and Harper (5) reported that the hyphae developed from fused sporidia in *Ustilago avenae* and *U. levis* and in *U. antherarum*, respectively, revert to the production of sporidia eventually. Sleumer (16) stated that the addition of nutrient material to cultures of *U. zeae* resulted in sporidial formation by both uninucleate and binucleate mycelia. The possible influence of adding nutrients to the cultures was not investigated by the writers. Hanging-drop cultures were maintained in the laboratory for 2 months, however, without any evidence of sporidial formation from binucleate hyphae. Frequently uninucleate hyphae were found to produce aerial sporidia at the surface of the culture drop. The binucleate hyphae varied considerably in size and often were no coarser than the uninucleate hyphae. Hence, it became necessary to trace any hypha in question to its origin or resort to staining procedure to determine its nuclear condition.

NUCLEAR BEHAVIOR AFTER FUSION

When sporidial cultures had reached the desired stage for staining, they were killed with Flemming's weaker solution or Carnoy's alcohol-acetic acid solution or simply by drying them rapidly over a low flame. When thoroughly dried, the cultures were found to adhere to the slide or cover slip sufficiently well to permit their manipulation in the staining procedure without appreciable loss.

The protoplasmic contents in the rapidly growing portions of both uninucleate and binucleate hyphae exhibited a marked affinity for certain dyes and destained very irregularly. This was especially true when crystal violet was used. Either Heidenhain's or Delafield's haematoxylin gave greater uniformity. The latter stain was more satisfactory because the cell walls of the preparations thus stained were more readily discernible. A weak concentration of acid fuchsin in

lactophenol was found to be fairly satisfactory as a general, semi-permanent, rapid stain.

The procedure followed with Heidenhain's iron alum haematoxylin was essentially the same as that described by Holton (6). The procedure employed with Delafield's haematoxylin was as follows: The preparation was dried, then fixed in dilute Carnoy's solution for 2 to 5 minutes; flooded with water, which was removed with filter paper or cotton swab; dried over a very low flame; stained 3 to 30 minutes; washed by flooding with water; destained in acid alcohol; transferred to 70-percent alcohol and dehydrated through the higher alcohols; and then cleared and mounted in Canada balsam.

The nuclear behavior and distribution of the protoplasmic contents of the fused sporidia and binucleate hyphae in *Ustilago maydis* were found to differ in some respects from the descriptions for other members of the Ustilaginaceae. Several investigators (1, 6, 8, 9, 11, 12, 13, 16) agree essentially that after fusion the protoplasmic contents of the fused sporidia migrate into the fusion hypha and thereby initiate the binucleate phase. The contents then continue to move toward the apex as the hypha elongates. Sleumer (16), Rodenhiser (13), and Holton (6), investigating *U. zeae*, *Sphacelotheca sorghi* and *S. cruenta*, and *U. avenae* and *U. levis*, respectively, showed the formation of cross walls in the basal portions of old binucleate hyphae, although each of the cells thus formed, as well as the fused sporidia, apparently was empty. Stakman, Cassell, and Moore (17) found essentially the same situation in *Urocystis occulta*. However, they occasionally observed two-celled fused sporidia in which the nucleus was present in the nonfused cell.

In the present study, definite, well-defined nuclei were observed frequently in the fused sporidia and in the older basal portions of the binucleate hyphae, but their occurrence was more or less sporadic. Some cells both of sporidia and of hyphae apparently still contained the normal protoplasmic contents, whereas in others the contents were in various stages of disintegration and some cells appeared to be empty. The representative nuclear condition of the fused sporidia and older portions of the binucleate hyphae are illustrated in figure 2. In figure 2, *A*, the direct H-shaped type of fusion between two sporidia has just occurred. Both sporidia are two-celled. A single nucleus is evident in each of the fused cells and also in one of the nonfused cells. This last cell has produced a uninucleate hypha, which would make it possible for the one sporidium to fuse with two different sporidia or for a double fusion between two sporidia to occur.

It was possible to follow the entire binucleate hypha that had developed from the fused sporidia shown in figure 2, *D*. Representative portions of this hypha are shown in *E*, *F*, and *G*. The protoplasmic contents of the cells in *D* apparently were disintegrating, although scattered nuclei were clearly visible. In the fourth cell from the point of fusion (*E*, *a*) no nuclei were evident and only one each was observed in the fifth (*E*, *b*) and sixth (*E*, *c*) cells. The distribution of the cytoplasm in all three cells was rather irregular. In the middle portion of this hypha, the sixteenth cell (*F*, *b*) contained two clearly defined nuclei. Only one nucleus was visible in the seventeenth cell (*F*, *c*), and none was visible in either the fifteenth (*F*, *a*) or the eighteenth (*F*, *d*) cell. The cytoplasm, although not so uneven or patchy as in the older cells, was nevertheless in sharp contrast to the regular dis-

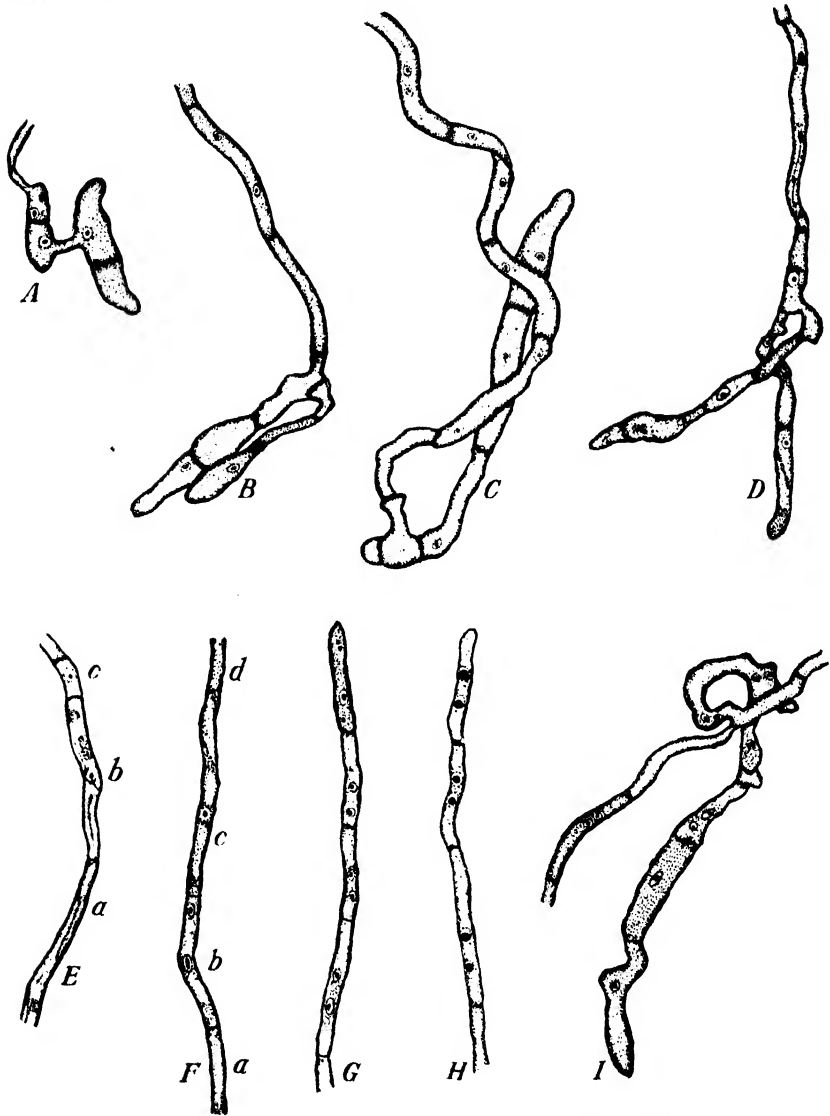


FIGURE 2.—Sporidial fusion and nuclear condition in *Ustilago maydis*. Approximately $\times 1,825$. A, An early stage in the direct H-shaped type of sporidial fusion. Both sporidia are two-celled. The binucleate hypha has not yet developed. B, Sporidial fusion by means of short, uninucleate hyphae, or germ tubes, developed from ends of compatible sporidia. Paired nuclei are visible in the second cell of the binucleate hypha. C, Sporidial fusion in which one cell of one sporidium has functioned as the fusion cell. Paired nuclei are shown in three cells of the binucleate hypha. D, Fused sporidia. E to G, Representative portions of the binucleate hypha developed from the fused sporidia in D. The irregular cytoplasmic and nuclear condition commonly found in fused sporidia and older portions of binucleate hyphae is shown in D, E, and F. G, Paired nuclei in the tip cell of the hypha. H, Paired nuclei in the three apical cells of a representative binucleate hypha. I, Irregular and branched binucleate hypha. A, B, C, and I, stained with acid fuchsin; D to G, with Delafield's haematoxylin; H, with orseiline BB and aniline blue. (See text, p. 238, for explanations of a, b, c, and d.)

tribution of that in the younger cells near the tip of the hypha, as shown in *G*. There was also a decided difference in the staining reaction of the older and younger portions of the hypha. The older cells that contained one or more nuclei each, although staining more densely than those in which no nuclei were apparent, did not retain as much of the stain as did the rapidly growing cells at the end of the hypha.

Sleumer (16) distinguished three and sometimes four nuclei in the tips of binucleate hyphae in *Ustilago zeae* but did not show pairs of nuclei separated by cell walls. Allison (1), reporting on *U. levis* and *U. hordei* (Pers.) Lagerh., pictured one hypha containing six nuclei and another containing four, but no septa in either. Stakman, Cassell, and Moore (17) found that the binucleate hyphae of *Urocystis occulta* often contain two pairs of nuclei, frequently three nuclei, but rarely a single nucleus. They assume that the fourth and the second nucleus, respectively, may not have been visible or that one nucleus had not yet divided.

In the present study, paired nuclei, clearly delimited by septa, have been commonly observed in the end cells of binucleate hyphae. Paired nuclei were commonly found in the two end cells and occasionally in as many as five contiguous cells at the end of a binucleate hypha. Representative examples are illustrated in figure 2, *G* and *H*. In *G*, two small, darkly stained bodies are just visible in the tip of the apical cell. Whether these represent two daughter nuclei produced by division prior to cell-wall formation could not be definitely determined. The preceding five cells of this hypha (not illustrated) were well stained, but no pairs of nuclei could be definitely determined. The nucleoli shown in the three end cells of another binucleate hypha (*H*) appear to be slightly larger than those in other illustrations. This may have been due to the use of orseilline BB and aniline blue in place of Delafield's haematoxylin in the staining procedure.

Occasionally irregularly formed and branched binucleate hyphae (fig. 2, *I*) were observed. Sleumer (16) described a somewhat similar condition, which he termed "Wirkopulation." Inasmuch as the binucleate hyphae become branched and irregular in form within the host tissues, the occurrence of this condition in culture would seem to be more or less expected and not an exception in need of an explanation. One cell apparently contains two pairs of nuclei. The failure of a cell wall to form might have caused this condition; or possibly, as suggested by the shape of the cell, a new branch was beginning to develop into which the nuclei would migrate.

DISCUSSION

That the binucleate stage in the smut fungi is initiated in culture by the fusion of basidiospores (sporidia) has been demonstrated for a number of species in the Ustilaginaceae. However, the ability of the sporidia of *Ustilago maydis* to fuse in culture has not been clearly established previously. In the present study the fusion of compatible sporidia was obtained in culture. Its occurrence was found to depend largely on nutrition and temperature. Both factors influenced the time required for fusion to take place. In certain other smuts fusion occurs in a relatively short period of time. Holton (6) obtained fusion in *U. avenae* and *U. levis* after 35 minutes to 4 hours. Stakman,

Cassell, and Moore (17) observed fusion between the sporidia of *Urocystis occulta* as soon as the sporidia were fully formed. Rodenhiser (13) observed fusion in *Sphacelotheca sorghi* and *S. cruenta* after 10 hours. In the present study, however, fusion was first detected after 15 to 20 hours and was not readily observed until after 40 to 48 hours. The relatively long period of time and the extremely low concentration of nutrients, which apparently are necessary for sporidial fusion, may account for the negative results of many previous investigators.

From the present investigation of *Ustilago maydis* it could be deduced that the general nuclear behavior might be as follows. Simultaneously with, or immediately after, conjugation, the nucleus in each of the fusing gametes divides and a daughter nucleus migrates into the fusion tube or cell. Cell division is completed in the normal manner by means of cell-wall formation. Further growth of the binucleate hyphae is accomplished by normal cell division, i. e., nuclear division, migration of daughter nuclei, and cell-wall deposition. Each cell of the binucleate hyphae, therefore, normally contains one pair of nuclei.

The gradual aging and decline of the cells in the older portion of the hypha simultaneously with the growth of the apical region suggest that a disintegration of the protoplasmic contents of the older cells occurs at a rate depending upon the environmental conditions present in artificial culture. The extent to which this process is influenced by any particular set of environmental or nutritional conditions was not determined. The existence of physiological or biochemical differences between different portions of the hyphae is indicated by the differences in dye absorption and retention. Further studies might indicate a situation somewhat analogous to that described by Thomas (18) for several species of *Pythium*, in which he found that the fixation of dyes is determined by differences in cell-wall composition of young and that of old, mature hyphae. Whether the cells appear devoid of contents or contain nuclei either singly or in pairs would depend, therefore, upon the degree of protoplasmic aging and disintegration as well as upon the success of the staining technique.

If this interpretation is correct, the presence of empty fused sporidia and empty cells in old portions of binucleate hyphae or the appearance of cells with irregular numbers of nuclei might better be explained on the basis of physiological aging and disintegration of cell contents than of an irregular or unusual type of nuclear and cellular division. The viewpoint frequently expressed in the literature that the protoplasmic contents of fused sporidia pass into the fusion hypha and migrate toward the tip as growth occurs, leaving empty cells behind, was not found in this study to be applicable to *Ustilago maydis*.

SUMMARY

The production and fusion of sporidia in cultures of *Ustilago maydis* are described. The initiation of the binucleate stage by the fusion of compatible sporidia is described and illustrated. The fusion process was influenced markedly by the nutritive value of the culture media and by the incubation temperature.

Fused sporidia were sufficiently numerous to be readily detected after 20 hours at 24° C. in distilled water and after 15 to 20 hours at 20° to 24° in 1-percent malt-extract solution.

The initiation of the binucleate phase followed no rigidly fixed method but resulted from the fusion of any two compatible haploid cells, either sporidial or hyphal. In no case were the binucleate hyphae observed to revert to sporidial production.

End cells of binucleate hyphae uniformly contained one pair of nuclei each. The protoplasmic contents of the older binucleate cells and of the fused sporidia appeared to be in various stages of disintegration. In some cases the cell contents were apparently normal, whereas in others they were either partly disintegrated or entirely lacking.

The rapidly growing apical cells of binucleate hyphae and young sporidia exhibited a pronounced affinity for the several dyes used, whereas older cells and old fused sporidia did not.

Since the results presented are based on a study of the fungus in culture they may or may not represent the situation as it exists in nature in the corn plant.

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UTILIZATION OF NITRATES BY THE COFFEE PLANT UNDER DIFFERENT SUNLIGHT INTENSITIES ¹

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INTRODUCTION

In the course of an investigation (7) ³ dealing with the levels of nitrogen, potassium, and carbohydrates in the leaves of coffee trees (*Coffea arabica* L.) grown at Kona, T. H., it was found that the leaves of young coffee trees (2 to 10 months old) accumulated relatively large amounts of nitrate nitrogen as compared with the leaves of other plants, and that the nitrate content fluctuated widely during the growing season. No adequate explanation for this seemingly unusual behavior was found. For this reason a study was made to determine the cause or causes which lead to the accumulation of nitrates in coffee leaves. After a consideration of the important external factors that influence nitrate metabolism, it appears that at Kona sunlight intensity might be the limiting factor in the process; hence, in this investigation the chief emphasis was placed on the effect of different degrees of sunlight intensity on the assimilation of nitrates by the coffee plant. In order to maintain strict control over the nutrient supply, the coffee plants were grown in water culture. This paper presents the results of the investigation.

REVIEW OF LITERATURE

A review of the factors that play a dominant role in the nitrate nutrition of green plants has been published by Nightingale (21).

The nitrate content of plants varies with different treatments and with the stage of growth (1, 4, 6, 9, 35). When the external supply of nitrate is plentiful many plants store large amounts in their roots, stems, or leaves without injurious effects (1, 4, 6, 19, 35, 40). Nitrates are known to accumulate in plants when carbohydrate synthesis is curtailed or when sunlight intensity is reduced (11, 32, 33), when temperature is low (20, 23, 25), and when potassium (22, 26-34) phosphorus (8, 10, 34), or manganese (17, 39) is deficient. Under conditions of high concentration of salts in the culture solution, the reduction and assimilation of nitrates within the plant become somewhat limited (24). Plants growing under low soil moisture conditions have also been found to accumulate nitrates (12).

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³ Italic numbers in parentheses refer to Literature Cited, p. 256.

EXPERIMENTAL METHODS

The coffee seeds used in this study were obtained from a single tree at Kona, Hawaii. After the seedlings had grown for 3 weeks in a very dilute nutrient solution, two seedlings were transferred to each of a number of gallon jars containing the complete nutrient solution. The solutions were changed once every 3 weeks. The culture solutions were aerated moderately and continuously, and their reaction was maintained between pH 4.5 and pH 6.5. The composition of the complete nutrient solution was as follows: ⁴

KNO₃—0.0012 M

Ca (NO₃)₂—0.0012 M

KH₂PO₄—0.00025 M

MgSO₄·7H₂O—0.0012 M

5 cc. of a 2-percent stock solution of ferric tartrate.

5 cc. from a liter of solution containing 0.25 gm. H₃BO₃, 0.5 gm. MnCl₂·4H₂O, 0.25 gm. ZnSO₄, 0.1 gm. CuSO₄·5 H₂O.

On February 24, 1943, 44 plants were placed in the open, 24 plants were placed under moderate shade, and 24 plants under heavy shade. The moderate shade consisted of laths 2.5 cm. in width spaced 2.5 cm. apart, and the heavy shade consisted of 5.0-cm. laths spaced 2.5 cm. apart. After 3 weeks, the width of the laths of the heavy shade was increased to 7.5 cm., but the spacing was kept at 2.5 cm. Hereafter in this paper the moderate shade is referred to as one-half shade and the heavy shade as three-fourths shade.

Growth measurements and sunlight radiation records were made weekly, beginning on February 24. Sunlight intensity was measured by the decomposition of oxalic acid in 0.01 M uranyl sulfate solution. It has been found that reliable sunlight measurements can be obtained by the use of this method.⁵

TABLE 1.—Outline of treatment of coffee plants in experiment begun Feb. 24, 1943

Experiment No. and series	Number of plants	Treatment
1:		
A	8	Complete nutrient; no shade.
B	6	Complete nutrient; one-half shade.
C	6	Complete nutrient; three-fourths shade.
2:		
A 1	8	3 weeks before sampling —N; no shade.
B 1	6	3 weeks before sampling —N; one-half shade.
C 1	6	3 weeks before sampling —N; three-fourths shade.
3:		
A 1	8	3 weeks —N followed by 3 weeks +N; no shade.
B 1	6	3 weeks —N followed by 3 weeks +N; one-half shade.
C 1	6	3 weeks —N followed by 3 weeks +N; three-fourths shade.
4:		
A 2	8	6 weeks before sampling —K; no shade.
B 2	6	6 weeks before sampling —K; one-half shade.
C 2	6	6 weeks before sampling —K; three-fourths shade.
5:		
A 3	6	6 weeks before sampling —P; no shade.
6:		
A 4	6	6 weeks before sampling +2 N; no shade.

¹ KNO₃ and Ca (NO₃)₂ were replaced by equimolar concentrations of KCl and CaCl₂.

² KNO₃ and KH₂PO₄ were replaced by equimolar concentrations of NaNO₃ and NaH₂PO₄.

³ KH₂PO₄ was replaced by an equimolar concentration of KCl.

⁴ Preliminary investigations showed that best growth was made in solutions of low osmotic pressure.

⁵ From personal correspondence with H. W. Brodie of the Hawaiian Sugar Planters' Association. Honolulu, T. H. 1942.

On June 30, approximately 27 weeks after germination, the coffee plants were divided into six series and the treatments shown in table 1 were begun. After 6 weeks of these treatments, mature leaves from all plants were sampled. The fresh leaves were weighed, dried under strong draft in an electric oven at 80°–90° C., and ground to pass a 40-mesh screen.

In order to see whether similar results would be obtained during the cooler months of the year, the entire experiment was repeated with the same plants. On October 5 one plant was placed in each jar, and thereafter the solution was changed once every 2 weeks. On December 16, except for the plants in the nitrogen series, the plants were given the treatments mentioned previously. The plants in the nitrogen series were without nitrogen for 4 weeks. On January 27, 1944, leaves were sampled and treated in the same manner as before.

ANALYTICAL METHODS

Total nitrogen was determined by the Kjeldahl method after preliminary reduction with reduced iron powder (27).

Soluble nitrogen was extracted from the dried tissue with distilled water (37) and was determined as total nitrogen.

Insoluble nitrogen was determined by subtracting soluble nitrogen from total nitrogen.

Nitrate nitrogen was determined in an aliquot of the soluble nitrogen extract by reduction with Devarda's alloy.

Ammonia and amide nitrogen were analyzed in an aliquot of the soluble nitrogen extract by the method of Pucher et al. (28).

Alpha-amino nitrogen was determined in the residue from the ammonia determination by the Van Slyke method (18).

Peptide nitrogen was analyzed in an aliquot of the soluble nitrogen extract by the acid hydrolysis method of Vickery et al. (36).

Basic nitrogen was determined in an aliquot of the soluble nitrogen extract with phosphotungstic acid as the precipitant.

Soluble sugars were extracted with hot 80-percent alcohol, and after hydrolysis with invertase (18), were determined by the method of Quisumbing and Thomas (3).

Starch was determined in the residue from the alcohol extract (18).

Hemicellulose was determined in the residue from the starch determination (18).

Potassium was determined by the Na-cobaltinitrite method of Volk and Truog (38). The tissues were wet-ashed with a nitric-perchloric acid mixture.

Phosphorus and calcium were analyzed in the wet-ashed tissues by standard methods.

EXPERIMENTAL RESULTS

APPEARANCE OF PLANTS

The appearance of the coffee plants was greatly affected by the different light treatments. This can be seen in figure 1, where photographs are shown of typical plants from each light treatment.

Contrary to the results obtained by some others (2, 29, 31), who have investigated the effects of shading on the coffee plant, no ill effects from strong sunlight were manifested by the unshaded plants despite their gross structural differences from the shaded plants.

Some of the morphological differences arising from differences in shading were similar to those previously reported. Thus, shading increased the length of internodes (2, 29, 30) and size of leaves (2, 13, 29, 30) but decreased the size of the root system (14) and the trunk. There were more leaves per plant in the unshaded series than in either of the shaded series. Plants grown under three-fourths shade appeared rather spindling and had the smallest number of leaves per plant. In most respects, the plants grown under one-half shade

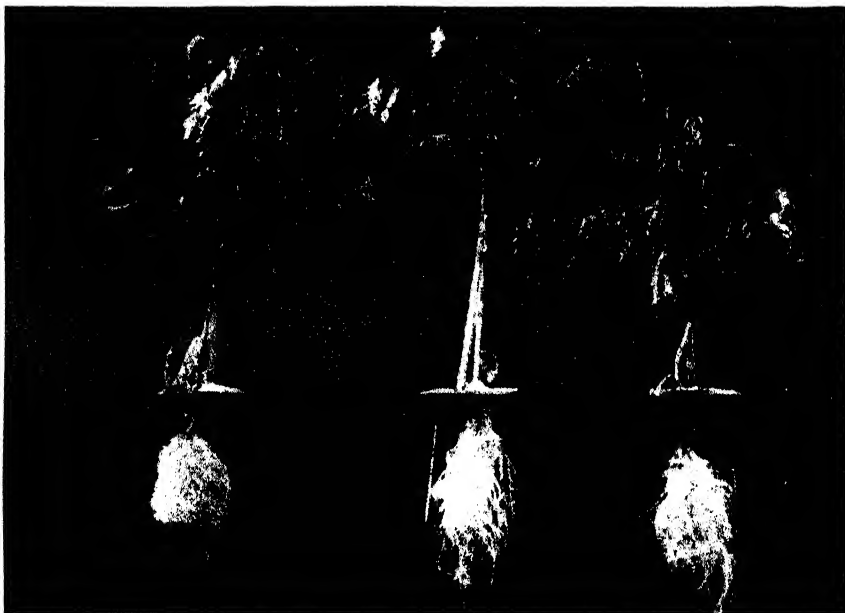


FIGURE 1.—Coffee plants grown under three different light conditions. A, No shade; B, one-half shade; and C, three-fourths shade.

occupied an intermediate position between those grown without shade and those grown under three-fourths shade.

In all the minus-nitrogen series (table 1, experiments 2 and 3) the unshaded leaves were yellowish green after the period during which they were without nitrogen. In the case of the minus-potassium series (experiment 4) the leaves of the plants grown without shade developed symptoms of potassium deficiency. Necrotic areas appeared along the margins of mature leaves and enlarged as the deficiency progressed. In figure 2 are photographs of three leaves showing various stages of potassium deficiency. The plants grown without phosphorus (experiment 5) showed no visible signs of its absence.

RELATION BETWEEN GROWTH AND SUNLIGHT INTENSITY

In many coffee-producing countries the coffee plant is grown under shade because of the general belief that shading is essential for the best growth of coffee plants, especially those of the Arabian type. Yet, as far back as 1901, after a canvass of the subject, Cook (5) came to the conclusion that there is no basis for the belief that shade is necessary for the growth of the coffee plant.

The growth of the coffee plants and the sunlight intensity during the first experiment are graphically depicted in figure 3. Sunlight radiation is expressed as milliequivalents of oxalic acid decomposed. From the curves for cumulative sunlight, it can be seen that the one-half shade series received only 40 percent of the full sunlight, while the three-fourths shade series received about 20 percent growth and sunlight records obtained during the second experiment were similar to those of the first experiment. However, the weekly sunlight intensity record of the second experiment was about 75 percent of that of the first experiment.

During the first half of the first experiment the growth of the plants under the three light conditions appeared to be similar, but toward the end of the second half of the experiment the growth of the plants without shade was better than that of the shaded series (fig. 3). To determine whether the difference in growth among the three series

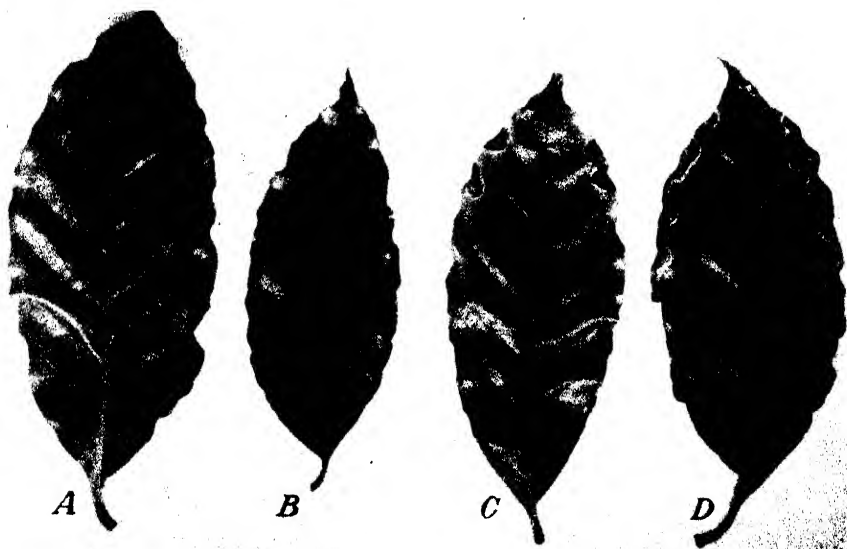


FIGURE 2.—A, Normal coffee leaf; B-D, coffee leaves showing progressively increasing severity of potassium deficiency.

were significant, the data were statistically examined by analysis of variance. In so doing, the growth period was divided into two parts—March 1 to May 3 and May 3 to June 28—and the increases in growth during the two periods were calculated. Table 2 contains the data showing the average increase in height during the two periods.

Statistical evaluation of the growth data showed that during the early stages of growth, the coffee plants grew better with half shade than with no shade (table 2), but during the latter half of the first experiment, the growth of the plants without shade was significantly better than that of the plants under one-half or three-fourths shade. The growth of the plants under one-half shade was also significantly better than that of the plants under three-fourths shade. When the growth data of the second experiment were evaluated, however, the results indicated that growth of the plants without shade was not

significantly better than that of the plants with one-half shade (table 2). Nevertheless, the growth of the unshaded plants and that of the one-half shade plants were significantly better than the growth of the three-fourths shade plants. These results and those of the first experiment indicate that the growth of the coffee plants was decreased by heavy shading.

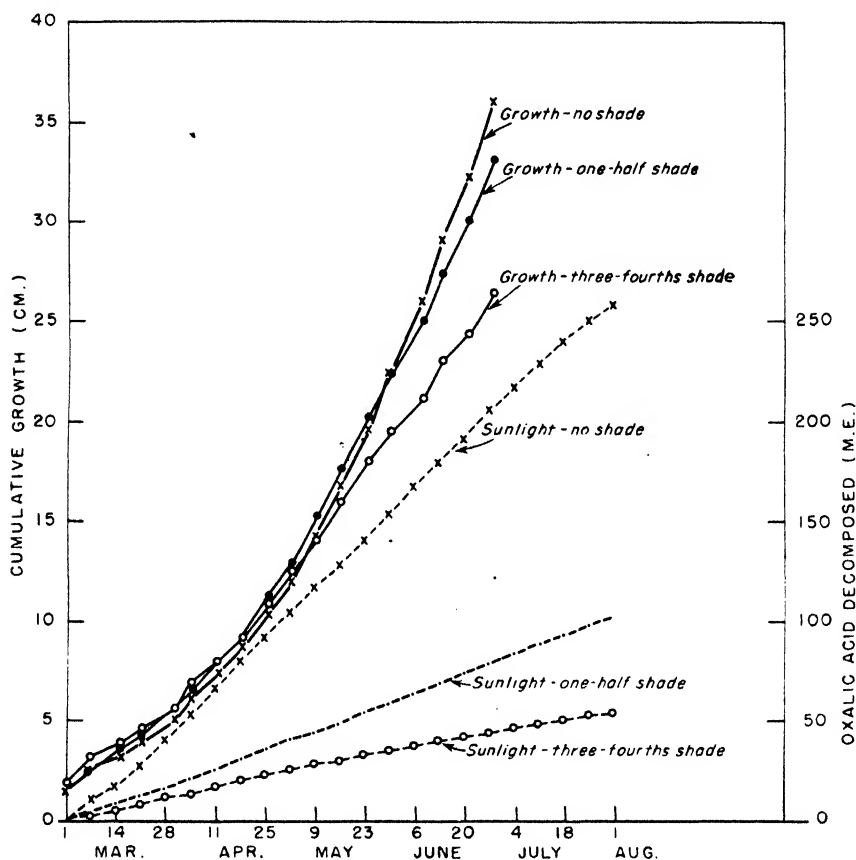


FIGURE 3.—Curves showing cumulative growth of coffee plants and intensity of sunlight during the growing period.

TABLE 2.—Average increase in height, during 3 growth periods, of coffee plants receiving no shade, one-half shade, and three-fourths shade, 1943

Series	Shade	Experiment 1		Experiment 2
		Mar. 1– May 3 ¹	May 3– June 28 ²	Oct. 5– Dec. 13 ³
X	None.....	Cm. 10.4	Cm. 24.1	Cm. 16.5
Y	One-half.....	11.6	20.0	14.5
Z	Three-fourths.....	10.6	13.9	10.0

¹ Difference necessary for significance $P=0.01$ —X-Y (or Z) ... 1.2 cm.; Y-Z ... 1.4 cm.

² Difference necessary for significance $P=0.01$ —X-Y (or Z) ... 1.9 cm.; Y-Z ... 2.1 cm.

³ Difference necessary for significance $P=0.01$ —X-Y (or Z) ... 3.8 cm.; Y-Z ... 4.4 cm.

The results obtained from the trunk measurements of the coffee plants seem to confirm the results obtained from the growth measurements. From table 3 it can be seen that unshaded plants were significantly larger in circumference than the plants of either shaded series. The circumferences of the one-half shade plants were also larger than those of the three-fourths shade plants.

CHEMICAL ANALYSES OF COFFEE LEAVES

The results of the chemical analyses of the coffee leaves are recorded in tables 4 to 7.

Dry-matter content.—Although the dry-matter content of the leaves differed somewhat in the different light series, dry matter was decreased by shading in all series (table 4). Severe potassium deficiency resulted in an increase in dry-matter content (experiment 8), probably because of the necrotic tissues.

TABLE 3.—Average circumference of trunks of coffee plants receiving no shade, one-half shade, and three-fourths shade

Series	Shade	Circumference ¹
		Cm.
X	None	4.5
Y	One-half	3.5
Z	Three-fourths	2.8

¹ Difference necessary for significance $P=0.01$ X-Y (or Z) . . . 0.2 cm.; Y-Z . . . 0.2 cm.

TABLE 4.—Moisture content of leaves from coffee plants receiving no shade, one-half shade, and three-fourths shade, with and without complete nutrient

[On green-weight basis]

Experiment No. and series	Shade	Nutrient	Moisture	Dry matter
			Percent	Percent
1: A	None	Complete	72.83	27.17
B	One-half	do.	74.35	25.65
C	Three-fourths	do.	75.35	24.65
2: A	None	—N, 3 weeks	73.39	26.61
B	One-half	do.	74.50	25.50
C	Three-fourths	do.	75.00	25.00
3: A	None	—N, 3 weeks; +N, 3 weeks	72.58	27.42
B	One-half	do.	73.30	26.70
C	Three-fourths	do.	74.10	25.90
4: A	None	—K, 6 weeks	71.00	29.00
B	One-half	do.	72.84	27.16
C	Three-fourths	do.	74.00	26.00
5: A	None	—P, 6 weeks	72.57	27.43
6: A	do.	+2 N, 6 weeks	71.42	28.58
7: A	do.	Complete	67.51	32.49
8: A	do.	—K	65.23	34.77
9: A	do.	—P	68.98	31.02

¹ Plants used in preliminary experiment. They were given treatment indicated until definite deficiency symptoms appeared.

Total nitrogen.—Total nitrogen increased with shading (table 5, experiment 1). Withholding nitrogen from the plants resulted in a decrease in the total nitrogen content and reduced the differences in nitrogen content existing among the plants under different light treat-

ments (experiment 2). These results showed that a good proportion of the nitrogen in the shaded plants was storage nitrogen. As expected, increasing the external nitrogen supply increased the total nitrogen content of the leaves.

TABLE 5.—*Nitrogen in leaves of coffee plants receiving no shade, one-half shade, and three-fourths shade, with and without complete nutrient*

[On dry-weight basis]

Experiment No. and series	Shade	Nutrient	Total N	Soluble N	Insoluble N	Nitrate N	Basic N
			<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
1:							
A	None	Complete	2.72	0.772	1.95	0.027	0.514
B	One-half	do	3.18	.895	2.28	.123	.520
C	Three-fourths	do	3.44	.971	2.47	.230	.494
2:							
A	None	-N, 3 weeks	2.51	.705	1.80	.019	.499
B	One-half	do	2.56	.679	1.88	.024	.480
C	Three-fourths	do	2.66	.680	1.98	.057	.472
3:							
A	None	-N, 3 weeks, +N, 3 weeks.	2.44	.663	1.78	.015	.464
B	One-half	do	2.59	.621	1.97	.045	.444
C	Three-fourths	do	3.04	.777	2.26	.105	.445
4:							
A	None	-K, 6 weeks	2.78	.821	1.96	.021	.502
B	One-half	do	3.02	.804	2.22	.053	.495
C	Three-fourths	do	3.32	.949	2.37	.078	.476
5	None	-P, 6 weeks	2.98	.830	2.15	.063	.525
6	do	+2 N, 6 weeks	3.47	1.193	2.28	.143	.623
7 ¹	do	Complete	2.70	.715	1.98	.031	.483
8 ¹	do	-K	2.72	.878	1.84	.035	.508
9 ¹	do	-P	3.77	1.921	1.85	.214	.773

¹ Plants used in preliminary experiment. They were given treatment indicated until definite deficiency symptoms appeared.

Severe phosphorus deficiency (table 5, experiment 9) resulted in an abnormal accumulation of nitrogen. The figures for total and soluble nitrogen showed that this increase was due to an abnormal increase in soluble nitrogen.

Potassium deficiency did not seem to affect the total nitrogen content of the leaves.

Soluble nitrogen.—With an ample supply of nitrogen, soluble nitrogen increased with shading (table 5, experiment 1). The increase was largely due to the accumulation of nitrates. When nitrogen was withheld from the plants (experiment 2) there was a decrease in soluble nitrogen. During the second experiment the leaves of the unshaded plants tended to have less soluble nitrogen than those of the shaded plants.

Lack of potassium increased the soluble nitrogen content of the leaves to some extent, owing largely to an increase in the basic nitrogen fraction.

A severe lack of phosphorus (experiment 9) resulted in an abnormal increase in soluble nitrogen, chiefly due to increases in the amounts of nitrate and peptide nitrogen. Basic nitrogen and alpha-amino nitrogen were also accumulated by the phosphorus-starved plants.

Insoluble nitrogen.—Insoluble nitrogen increased with shading and decreased when the external nitrogen supply was cut off (experiments 1 and 2, table 5). Hopkins (15) and Kraybill (16) have reported similar results for other plants.

Nitrate nitrogen.—The amount of nitrate nitrogen in the leaves varied widely in the first experiment, ranging from a few thousandths

of 1 percent to over two-tenths of 1 percent (table 5). In the second experiment there was less nitrate nitrogen in the leaves, probably because they were more mature.

Although sunlight is known to influence the assimilation of nitrate by green plants (21), the low percentages of nitrate nitrogen in the leaves of plants grown without shade point to the relative unimportance of sunlight in the assimilation of nitrate nitrogen by coffee leaves. Nitrate nitrogen increased with increase in shading as well as with increase in the external supply. When there was no nitrate in the culture solution, the nitrate stored in the leaves was quickly used up by the plant (experiment 2). On the other hand, when it was again supplied to the plant, the excess was again stored by the plant (experiment 3). These results indicate that when there is an excess of nitrate the accumulation of nitrate by coffee leaves under adequate nutritional conditions is a normal phenomenon; and the degree of accumulation is largely determined by the amount of solar energy available to the plants.

Phosphorus starvation resulted in an abnormal accumulation of nitrates. Other workers (8, 10, 34) have reported similar results with other plants. Eckerson (10) attributed the accumulation of nitrate to the role played by phosphorus in reductase synthesis.

Ammonia and amide nitrogen.—From the analytical results, it appears that ammonia nitrogen and amide nitrogen were not accumulated to any large extent by the coffee leaves under the conditions of this experiment. The figures ranged from less than 1 p. p. m. to 13 p. p. m. The trend of the figures indicates that both types of nitrogen are found in larger amounts in shaded leaves. Both forms seemed to accumulate when the nitrogen supply was greater than the utilization. The lack of phosphorus resulted in increases in ammonia nitrogen and amide nitrogen.

Basic nitrogen.—The high percentage of basic nitrogen (table 5) in the leaves of coffee plants indicates that this nitrogenous fraction cannot be overlooked in a consideration of the soluble nitrogenous constituents of coffee leaves. Under normal conditions it often comprises more than half of the total soluble nitrogen fraction. Cutting off the external supply of nitrogen resulted in a small decrease in its amount. Severe potassium deficiency caused some accumulation of basic nitrogen at the expense of insoluble nitrogen. Lack of phosphorus also resulted in accumulation of basic nitrogen.

Alpha-amino nitrogen.—In the first experiment the alpha-amino nitrogen showed a tendency to increase with shading. In the second experiment, however, this trend was not observed. The amount of alpha-amino nitrogen ranged from 0.03 percent to 0.11 percent. Lack of potassium did not seem to affect the concentration of alpha-amino nitrogen, but the absence of phosphorus (experiment 9) resulted in a large increase in the level of this constituent.

Peptide nitrogen.—The behavior of the peptide nitrogen fraction was somewhat similar to that of alpha-amino nitrogen. The peptide nitrogen in the coffee leaves ranged from 0.01 percent to 0.21 percent. Phosphorus deficiency resulted in an accumulation of this constituent.

Soluble sugars.—In the summer experiment the soluble sugar content showed a decided decrease with increase in shading (table 6, experiment 1), but in the winter experiment the trend was less evident. There was some accumulation of soluble sugars when potassium was

omitted from the culture solution for 6 weeks (experiment 4), but under conditions of severe deficiency of potassium (experiment 8), soluble sugars did not accumulate in the leaves. Nightingale (26) also found that at the outset of potassium deficiency, carbohydrates accumulate; and, with further lack of potassium, they decrease.

Starch.—The starch content of the leaves showed a very marked decrease with increase in shading (table 6). The starch content of the leaves in experiment 2 was much higher than that of experiment 1; nevertheless, it also showed a strong tendency to decrease with shading.

Hemicellulose.—In experiments 1 to 4 the hemicellulose fraction showed a tendency to increase with increasing shade (table 6).

Potassium.—In all light treatments (table 7, experiments 1 to 4) the potassium content of the leaves increased with an increase in shading. This is believed to be the result of luxury consumption. When the percentage of potassium in leaves dropped below about 2 percent, necrotic areas appeared along the leaf margin.

Calcium.—The calcium content of the leaves in both experiments 1 and 2 increased with shading (table 7).

TABLE 6.—Carbohydrates in leaves of coffee plants receiving no shade, one-half shade, and three-fourths shade, with and without complete nutrient

[On dry-weight basis as dextrose]

Experiment No. and series	Shade	Nutrient	Soluble sugars	Starch	Hemicellulose
			Percent	Percent	Percent
1: A	None	Complete	6.23	3.71	7.38
B	One-half	do	5.83	.32	7.65
C	Three-fourths	do	5.05	.16	8.30
2: A	None	—N, 3 weeks	6.65	4.60	6.98
B	One-half	do	6.42	2.88	8.35
C	Three-fourths	do	5.48	1.28	8.80
3: A	None	—N, 3 weeks; +N, 3 weeks	5.60	2.18	7.65
B	One-half	do	5.95	1.46	7.80
C	Three-fourths	do	5.42	.10	8.48
4: A	None	—K, 6 weeks	8.55	4.02	7.35
B	One-half	do	8.02	.16	8.20
C	Three-fourths	do	6.10	.16	8.00
5: A	None	—P, 6 weeks	6.42	1.24	6.90
6: A	do	+2N, 6 weeks	5.22	.16	6.88
7 ¹ : A	do	Complete	5.48	.34	6.88
8 ¹ : A	do	—K	5.52	.16	7.00
9 ¹ : A	do	—P	4.25	.16	6.98

¹ Plants used in preliminary experiment. They were given treatment indicated until definite deficiency symptoms appeared.

Phosphorus.—The results of experiments 1 and 2 (table 7) indicated that the phosphorus content of the coffee leaves was slightly reduced by the light treatment.

The behavior of the phosphorus-deficient plants, as revealed by the results of the chemical analysis for the various nitrogenous fractions, showed that lack of phosphorus caused a very serious break-down in the normal process of nitrogen metabolism. The break-down seemed to be associated with the anabolic process of nitrogen metabolism.

TABLE 7.—Ash constituents in leaves of coffee plants receiving no shade, one-half shade, and three-fourths shade, with and without complete nutrient

[On dry-weight basis]

Experiment No. and series	Shade	Nutrient	Potassium	Calcium	Phosphorus
			Percent	Percent	Percent
1:					
A	None	Complete	2.89	1.46	0.20
B	One-half	do	3.40	1.60	.18
C	Three-fourths	do	4.06	1.66	.18
2:					
A	None	—N, 3 weeks	2.84	1.40	.20
B	One-half	do	3.10	1.53	.20
C	Three-fourths	do	3.52	1.58	.19
3:					
A	None	—N, 3 weeks; +N, 3 weeks	3.06	1.40	.20
B	One-half	do	3.58	1.56	.20
C	Three-fourths	do	4.06	1.58	.19
4:					
A	None	—K, 6 weeks	1.62	1.50	.18
B	One-half	do	1.82	1.58	.18
C	Three-fourths	do	2.49	1.80	.21
5:					
A	None	—P, 6 weeks	3.20	1.47	.10
6	do	+2N, 6 weeks	3.46	1.72	.20
7	do	Complete	2.74	1.35	.15
8	do	—K	.76	1.52	.16
9	do	—P	2.84	1.23	.06

† Plants used in preliminary experiment. They were given treatment indicated until definite deficiency symptoms appeared.

SUMMARY AND CONCLUSIONS

Coffee plants (*Coffea arabica* L.) were grown in water culture under three different sunlight intensities. The first study was conducted during the summer, the second during the winter. On the whole, the results obtained during the two seasons were about the same.

Under the conditions of this experiment, with height and trunk measurements used as indices, the coffee plants were found to grow better without shade than with heavy shade.

Plants grown without shade and under one-half shade appeared much hardier than those grown under three-fourths shade. The number of leaves per plant decreased with increase in shading, while the leaf size increased with shading. Unshaded plants had larger trunks and larger root systems than the shaded plants.

Chemical analyses of the leaves showed that increases in shading resulted in increases in total nitrogen, soluble nitrogen, insoluble nitrogen, ammonia nitrogen, amide nitrogen, nitrate nitrogen, alpha-amino nitrogen, peptide nitrogen, and hemicellulose; and resulted in decreases in dry matter, soluble sugars, and starch. Potassium and calcium tended to increase with shading. During the cooler period, phosphorus also tended to increase with shading.

Severe potassium deficiency resulted in an increase in the soluble nitrogen content. The increase was due principally to an increase in the basic nitrogen fraction. At the outset of potassium deficiency, soluble sugars accumulated but later fell back to normal values.

Severe phosphorus deficiency resulted in abnormal increases in total nitrogen, ammonia nitrogen, amide nitrogen, nitrate nitrogen, basic nitrogen, alpha-amino nitrogen, and peptide nitrogen. These results pointed to a serious break-down in the nitrogen metabolism of phosphorus-starved plants. The break-down seemed to be associated

with the anabolic process. Of the soluble nitrogenous fractions, nitrate nitrogen showed the greatest relative increase.

It is concluded that under suitable nutritional conditions, the accumulation of nitrates in the leaves of the coffee plant is a normal process, and that the amount stored at a certain period is determined largely by the amount of solar energy available to the plant at that time. If the supply of nitrate nitrogen is larger than the demand, the excess is stored by the plant.

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RELATION OF COLOR-INHIBITING FACTOR TO SMUDGE RESISTANCE IN ONION¹

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INTRODUCTION

The greater resistance of colored bulbs of onion (*Allium cepa* L.) than of white ones to the attack of the smudge organism (*Colletotrichum circinans* (Berk.) Vogl.) has long been known. The factors responsible for resistance have been given considerable study in recent years, chiefly with the object of developing resistant white varieties. The purpose of the study presented here was to determine the effect of different color genotypes upon resistance.

REVIEW OF LITERATURE

Berkeley (2),⁴ who first described onion smudge, observed that it was common on white bulbs but that colored ones were highly resistant. In 1921 Walker (10) gave an extensive description of the disease and its relation to environmental factors. In a series of studies Walker and associates (11, 12, 13, 14) showed that a water-soluble substance extracted from pigmented scales was toxic to the smudge organism and that this substance was not found in unpigmented scales. They showed also that the volatile substances present in onions were toxic, killing ungerminated spores and checking the growth of the mycelium. Later Walker and associates (1, 6, 7, 15) demonstrated that one of the toxic substances present in pigmented scales was protocatechuic acid. Link and Walker (8) determined further that catechol, which was present in pigmented scales and absent from white scales, was even more toxic than protocatechuic acid to the onion smudge organism. Ingersoll, Vollrath, Scott, and Lindegren (4) found that the volatile substances from freshly crushed onion and garlic contained unsaturated aldehydes, which they suspected to be either allyl aldehyde (acrolein) or crotonaldehyde, both of which were shown to have high bactericidal activity.

The genetic factors responsible for pigmentation in the onion were first described by Rieman (9), who demonstrated the occurrence of two kinds of factors for white, one a dominant inhibitor and the other

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⁴ Italic numbers in parentheses refer to Literature Cited, p. 264.

a recessive white factor. Later studies by Clarke, Jones, and Little (3) confirmed these results but showed that the factors differentiating red and yellow pigments were not allelomorphic with the recessive white factor, as postulated by Rieman (9), but were independent of it.

The dominant allele *C* is necessary for color development. A variety of onion homozygous for red has the genotype *iiCCRR*, for yellow *iiCCrr*, and for recessive white *iiCCRR*, *iiCCRr*, or *iiCCrr*. The *I* gene is incompletely dominant in the heterozygous condition, but all bulbs homozygous for *II* are white (3, 9).

MATERIALS AND METHODS

Several F_1 plants from each of six different crosses between white and yellow varieties were grown in the greenhouse at Beltsville, Md. The flowers were selfed, and the resulting seed was sent to Wisconsin for growing and testing. In addition, F_2 plants from a cross between Crystal Wax and Italian Red were selfed, and this seed, as well as that from the selfed white and colored parents, was sent to Wisconsin. At first it was thought that the Crystal Wax plant carried the recessive white factor *c* and the recessive allele of the dominant white factor *I*. The F_3 seed was, therefore, included for comparing the recessive white with the dominant white plants from other populations. However, it was discovered later that the Crystal Wax parent carried the dominant white factor and had the genetic constitution *IICC*, although most plants of this variety have the constitution *iiCC*. One of the crosses from which F_2 populations were grown had as the white parent a strain of Southport White Globe. The F_2 populations gave a segregation of 13 white and cream to 3 colored. It was therefore concluded that the white parent had the genetic constitution *Iicc*. Since the white bulbs appearing in such populations consist of five genotypes phenotypically indistinguishable, it was decided to omit all these populations from the analysis. In the remaining five crosses the variety White Portugal was used as the white parent. Most of these plants had the constitution *IICC* and gave ratios of 1 white to 2 cream to 1 colored in F_2 populations from crosses with colored (*iiCC*) plants. As one of the White Portugal lines, 3-213, had the genotype *Iicc*, all populations involving this parent were excluded from the analysis.

The colored parents involved in the crosses were Stockton G36, Italian Red, Early Grano, Stockton Yellow Flat, and Yellow Globe Danvers. The last-named variety is very pungent, whereas the other four are mild types.

Seed was sown in flats in the greenhouse in early March. Plants were transplanted outdoors in early May. Inasmuch as it was not possible to replicate each lot, plots of susceptible tester stocks of Southport White Globe and White Sweet Spanish varieties were distributed at random throughout the plots. The area in which the plots were located had also grown onions the 2 years immediately preceding. All these onion crops were inoculated with the smudge organism. Gross cultures of *Colletotrichum circinans* were grown on aerated Czapek's solution and distributed over the lower parts of the plants when the bulbs were about half-grown. Overhead irrigation

was applied during the succeeding night to provide favorable conditions for infection.

In late August when the tops were mature the bulbs were removed from the soil and placed in shallow crates in a protected outdoor location to cure. Curing was allowed to proceed in accordance with the usual commercial practice until mid-November, during which period the disease continued to develop and advance from the dry outer scales to the fleshy ones (10).

Disease analysis, made during the week of November 15, consisted first of dividing the bulbs into three color classes: White, cream, and yellow or red. The bulbs in each color class were then divided into four disease classes as follows:

- (0) No evidence of smudge.
- (1) Slight evidence of smudge, usually on the outermost scale only.
- (2) Moderate amount of smudge.
- (3) Large amount of smudge, with invasion of one or more fleshy scales.

The number of bulbs in each disease class was multiplied by the class number, and the sum (S) of the products was obtained. The index was then calculated by the following formula:

$$\frac{S \times 100}{3 \times \text{total population}} = \text{disease index.}$$

The uniformity of infection throughout the disease plot is shown by the disease indexes for the tester stocks, Southport White Globe and White Sweet Spanish. When distributed at random throughout the test plot, plantings of Southport White Globe gave index readings of 100.0, 95.4, 96.2, and 97.4; and White Sweet Spanish, 82.7, 77.4, 74.8, 61.3, and 92.2.

The analysis of variance was employed to obtain comparisons between the three genotypes II (white), Ii (cream), and ii (colored) and also between the different crosses. Only populations that showed a satisfactory fit to a 1:2:1 ratio were included in the analysis. As pointed out by Clarke, Jones, and Little (3), it is easy to distinguish white from cream bulbs in some populations, whereas in others the two classes cannot be separated with certainty. No doubt the populations that showed a poor fit to the expected 1:2:1 ratio contained many bulbs which had been incorrectly classified as to genotype. It was felt that the mean index for each group of bulbs within the populations was the most reliable measure of reaction to smudge; and, in order to avoid giving undue weight to occasional escapes or errors in classification, only those populations which had at least five plants in the smallest class were included in the analysis.

RESULTS

In calculating the variation due to genotype with respect to the I gene, it was found that in all six crosses there was a significant difference in susceptibility between genotypes, the F value always being several times that required for the 1-percent level of significance. However, this is to be expected on the basis of repeated observations by many workers that white bulbs are more susceptible to smudge than colored ones. The question that has not been answered satisfactorily heretofore is whether bulbs heterozygous for the I gene are

more resistant to smudge than the homozygous white ones. In all six of the crosses the mean index of the *Ii* bulbs showed that they were more resistant to smudge than the *II* bulbs. In four of these crosses the differences were highly significant. In the other two the differences were not significant because the number of populations was so small that large differences were required. In all six crosses the differences between the indexes of the colored bulbs (*ii*) and of the cream bulbs (*Ii*) were highly significant. In all but one cross, White Portugal \times Yellow Globe Danvers, the mean index of the heterozygous cream bulbs was slightly nearer the index of the white bulbs than that of the colored bulbs. When all crosses were considered, the mean index for all bulbs was 40.30 and that of the cream bulbs was 41.64. This small difference was not significant; so it can be concluded that from the standpoint of smudge susceptibility the *I* gene is scarcely, if at all, dominant over the *i* gene and that the heterozygous bulbs *Ii* are approximately intermediate between the two homozygous types in susceptibility.

The combined analysis of all the crosses (table 1) showed a difference due to genotypes and a highly significant difference between crosses, even though they all contained the same three genotypes as to color. It is evident, therefore, that factors besides color affect the susceptibility of the bulbs to smudge. The differences in susceptibility to smudge between crosses are shown in table 2.

TABLE 1.—Summary of data on smudge resistance in segregating progenies from 6 onion crosses

EXPERIMENTAL DATA

Cross	Populations	Disease indexes			
		<i>II</i>	<i>Ii</i>	<i>ii</i>	Mean
White Portugal \times Stockton G36.....	4	88.42	69.88	21.80	60.
Crystal Wax \times Italian Red.....	5	77.94	58.94	16.52	51.
White Portugal \times Early Grano.....	21	72.71	51.07	23.49	49.
White Portugal \times Italian Red.....	8	78.12	51.29	3.86	44.
White Portugal \times Stockton Yellow Flat.....	4	72.32	43.62	5.88	40.
White Portugal \times Yellow Globe Danvers..	21	46.01	18.66	5.98	23.
Mean ¹		65.89	41.64	13.38	40.

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	<i>F</i> values		
			Calculated	Required	
				5-percent level	1-percent level
Genotypes.....	2	43,505.90	310.22	3.08	4.80
Crosses.....	5	5,877.58	41.91	2.29	3.18
Populations (within crosses).....	57	410.44	2.93	1.45	1.68
Genotypes \times crosses.....	10	699.20	4.99	1.91	2.49
Error.....	114	140.24			

¹ Difference required for significance between genotype means at the 1-percent level=5.61.

TABLE 2.—Differences in susceptibility to onion smudge between crosses

[Basic data in table 1]

Cross	White Portugal × Stock- ton G36	Crystal Wax × Italian Red	White Portugal × Early Grano	White Portugal × Italian Red	White Portugal × Stock- ton Yel- low Flat	White Portugal × Yellow Globe Danvers
White Portugal × Stockton G36		8.90	10.94**	15.61**	19.42**	36.48**
Crystal Wax × Italian Red	8.90		2.04	6.71	10.52*	27.58**
White Portugal × Early Grano	10.94**	2.04		4.67	8.48*	25.54**
White Portugal × Italian Red	15.61**	6.71	4.67		3.81	20.87**
White Portugal × Stockton Yellow Flat	19.42**	10.52*	8.48*	3.81		17.06**
White Portugal × Yellow Globe Danvers	36.48**	27.58**	25.54**	20.87**	17.06**	

*Significant; **highly significant.

DISCUSSION

The fact that heterozygous cream bulbs are approximately intermediate in resistance between the pure white and the colored bulbs is important from the standpoint of breeding. It would be highly desirable to obtain varieties of white onions resistant to smudge, but if resistance is inevitably associated with the presence of pigment it would be difficult, if not impossible, to attain such an objective. However, cream bulbs with the genetic constitution *Ii*, though containing some pigment, might be as acceptable as pure white bulbs for certain purposes. The increase in resistance to smudge would probably more than compensate for the sacrifice in color purity. Although it would be impossible to obtain true breeding strains with the constitution *Ii*, the production of hybrid onions with this constitution would be very simple. This could be accomplished through the use of male-sterile strains as described by Jones and Clarke (5).

The effect of the recessive white gene *c* upon resistance to smudge has not been studied and will be the subject of further investigations. A difference between the susceptibility of red and yellow bulbs resulting from the action of the *Rr* pair of alleles has never been demonstrated and should be investigated further. Differences in susceptibility between crosses involving red and yellow parents did exist in the data presented here, but such differences are probably to be ascribed to factors other than color. It is noteworthy that the highest and lowest mean indexes of susceptibility were found in crosses between white and yellow varieties.

SUMMARY

The most important factor in resistance to onion smudge is the presence of pigment in the outer scales of the bulbs. White bulbs homozygous for the dominant color inhibitor *I* are highly susceptible to the disease. Colored bulbs with the genetic constitution *ii* are highly resistant. Cream bulbs with the constitution *Ii* are approximately intermediate in resistance between the pure white and the colored ones.

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DEVELOPMENT OF THE IMMATURE STAGES OF *ANASTREPHA SERPENTINA* IN RELATION TO TEMPERATURE¹

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INTRODUCTION

During the past several years large numbers of adults of *Anastrepha serpentina* (Wied.) have been found in the citrus groves of the lower Rio Grande Valley of Texas and Tamaulipas, Mexico. In 1934 James Zetek found sweet oranges purchased from the Panama City market to be infested with *A. serpentina*. In 1939 larvae were found in grapefruit at Mission, Tex., by employees of the Bureau of Entomology and Plant Quarantine. Hosts of economic importance in Mexico are chicozapote (*Achras zapota* L.), zapote mamey (*Calocarpum mammosum* (L.) Pierre), and peach (*Prunus persica* (L.) Stokes). The southern limit of *A. serpentina* given by Stone² is São Paulo in Brazil. In view of the threat of this insect to the citrus fruit industry a study of its development was undertaken at the Mexico City laboratory.

EGG STAGE

METHODS USED IN STUDY

Populations of fruitflies were reared from infested fruits of chicozapote and zapote mamey, but for the studies on the development of the species chicozapote and peach were used.

The flies to be used for supplying eggs were confined in glass-and-muslin cages, close to 12 inches in each dimension, and held in a large cabinet at 25° C. and approximately 60 percent relative humidity. Under these conditions the flies deposited the first eggs 16 days after emerging. The first mating was not observed until the twentieth day. Mating generally took place between 5 and 6 p. m. and lasted from 25 to 57 minutes.

The technique used for obtaining fruitfly eggs was that developed in Florida in 1929 during research on the Mediterranean fruitfly, *Ceratitis capitata* (Wied.) (unpublished). An outer section of chicozapote or peach was cut with the skin adhering to it, and most of the flesh was removed to leave a thin-walled shell (fruit skin), which the ovipositor of the fly could pierce. Each arc of fruit skin was fastened with paraffin to a 2½- by 2¾-inch glass plate. Since the eggs usually adhered to the inner side of the fruit skin, the laborious task of looking

¹ Received for publication August 24, 1944.

² STONE, A. THE FRUITFLIES OF THE GENUS *ANASTREPHA*. U. S. Dept. Agr. Misc. Pub. 439, 112 pp., illus. 1942.

for eggs in the flesh of whole fruits was thus avoided. The eggs, usually in groups of 4 to 12, could be easily observed through the glass. The fruit skin was removed from the glass when it became necessary to transfer the eggs.

The mounted fruit skins were placed in stock cages of flies for 2 or 3 hours between 1 and 7 p. m., the time of day when most oviposition by *Anastrepha serpentina* takes place.³ Eggs were removed from fruit skins by means of a camel's-hair brush and placed in rows on ink-blackened filter paper in a Syracuse watch glass. The filter paper was kept saturated with a 0.1-percent solution of cupric chloride, apparently without affecting the hatching of the eggs. Darby and Kapp⁴ used this solution to control the growth of fungi when they reared "pupae" of the Mexican fruitfly (*A. ludens* (Loew)) on cotton. The watch glasses were stacked and ground to fit tightly to avoid desiccation and the escape of tiny first instars. The largest number of eggs were deposited on fruit skins placed in a row along the cage floor against the glass front nearest the source of light.

From time to time equal numbers of fruit-skin sections of ripe chicozapote and peach were placed in stock cages of flies, and the eggs deposited in each kind of fruit were counted. Preference for chicozapote was shown when 599 eggs were laid in this fruit and only 151 in peach. When the mean difference of 15 comparisons was analyzed statistically, the indicated preference was shown to be highly significant. Chicozapote might well be expected to be the preferred host because it is attacked heavily by *Anastrepha serpentina* in the field.

The eggs were incubated in six insulated cabinets, each 32 by 13 by 15 inches. Temperature was controlled by using electric lamps as a source of heat, and ice to produce low temperatures. Shallow pans of water were placed in the cabinets at 25° C. or above to maintain a relative humidity of approximately 60 percent. Temperature was controlled within $\pm 0.15^\circ$ C. by means of toluene-mercury thermostats, and the air was circulated by 8-inch fans. A thermograph and a thermometer were kept in each cabinet. Care was taken to determine the temperature at the exact location of the biological material. At the end of the desired observation periods the containers with eggs were removed from the cabinets and quickly examined under a binocular microscope to record the hatch.

LENGTH OF INCUBATION AND HATCHING PERIODS

The incubation and hatching of eggs were studied at various temperatures between 10° and 37.8° C. The incubation period was reckoned from the time the fruit skins containing the eggs were transferred from the stock cages to the time of the last observation before the first egg hatched, and the hatching period from this observation until the last egg hatched.

When 1,000 eggs were held for 112 to 120 days at 10° C., no hatching was observed, although one-third to one-half of them appeared normal at the end of that time.

³ BAKER, A. C., STONE, W. E., PLUMMER, C. C., and MCPHAIL, M. A REVIEW OF STUDIES ON THE MEXICAN FRUITFLY AND RELATED MEXICAN SPECIES. U. S. Dept. Agr. Misc. Pub. 531, 155 pp., illus. 1944.

⁴ DARBY, H. H., and KAPP, E. M. OBSERVATIONS ON THE THERMAL DEATH POINTS OF *ANASTREPHA LUDENS* (LOEW). U. S. Dept. Agr. Tech. Bul. 400, 19 pp., illus. 1933.

At 12.5° C. none of 167 eggs hatched during a period of 81 days. After 72 days 1 larva partially emerged, and fully developed larvae were found in several eggs upon dissection. From equation 2, which is given later, it was calculated that hatching would be expected after 63 days.

No hatch was obtained from 699 eggs held at 35° and 572 eggs held at 37.8° C. Some of the eggs held at these temperatures became enlarged and split open longitudinally, exposing partially developed larvae, but more of the eggs simply collapsed. Other eggs remained white, turgid, and apparently normal after being held for 49 days at 37.8° C.

Eggs hatched at temperatures ranging from 15° to 32.5° C. A total of 3,520 eggs were incubated and observed at several temperatures within this range. Eggs held at 15° were observed usually twice each day, and those of one group (a) held at 25° were observed every 3 hours throughout the day and night. On eggs held at 20°, 30°, and 32.5°, and on a second group (b) at 25°, the time of observations was varied in an attempt to obtain points equally spaced in terms of the logarithm of time, according to the method used by Bliss.⁵ A summary of the incubation and hatching data obtained at the various temperatures is presented in table 1.

TABLE 1.—Summary of tests on incubation and hatching of *Anastrepha serpentina* eggs

Temperature (°C.)	Eggs		Incubation period	Hatching period
	Incubated	Hatched		
	Number	Percent	Hours	Hours
15.....	179	15.6	361.0	112.5
20.....	743	52.1	144.0	80.7
25 (a).....	368	21.5	90.0	39.0
25 (b).....	682	40.5	86.8	51.2
30.....	741	48.9	64.9	40.2
32.5.....	807	33.3	64.5	33.2

MATHEMATICS OF THE HATCHING PROCESS

A mathematical treatment of the hatching data was undertaken, and since satisfactory curves were fitted the essential details of the study will be described.

The asymmetric sigmoid curve obtained when the cumulative hatch was plotted against total developmental time suggested that it might be transformed to a straight line by converting the percentages of cumulative hatch to probits⁶ and the time units to logarithms. The straight-line relationship was not obtained, however, until a time constant was subtracted from the total developmental time. The incubation period was used as the constant.

The introduction of the time constant into the logarithmic term to fit the probit data to a straight line is in harmony with the use of

⁵ BLISS, C. I. THE CALCULATION OF THE TIME-MORTALITY CURVE. *Ann. Appl. Biol.* 24: 815-852, illus. 1937.

⁶ BLISS, C. I. THE CALCULATION OF THE DOSAGE-MORTALITY CURVE. *Ann. Appl. Biol.* 22: 134-167, illus. 1935.

similar constants by other workers. Bliss⁷ introduced a "threshold concentration" to straighten dosage-mortality curves, and Starr (unpublished reports) used an "approach-period equivalent" to produce straight mortality curves.

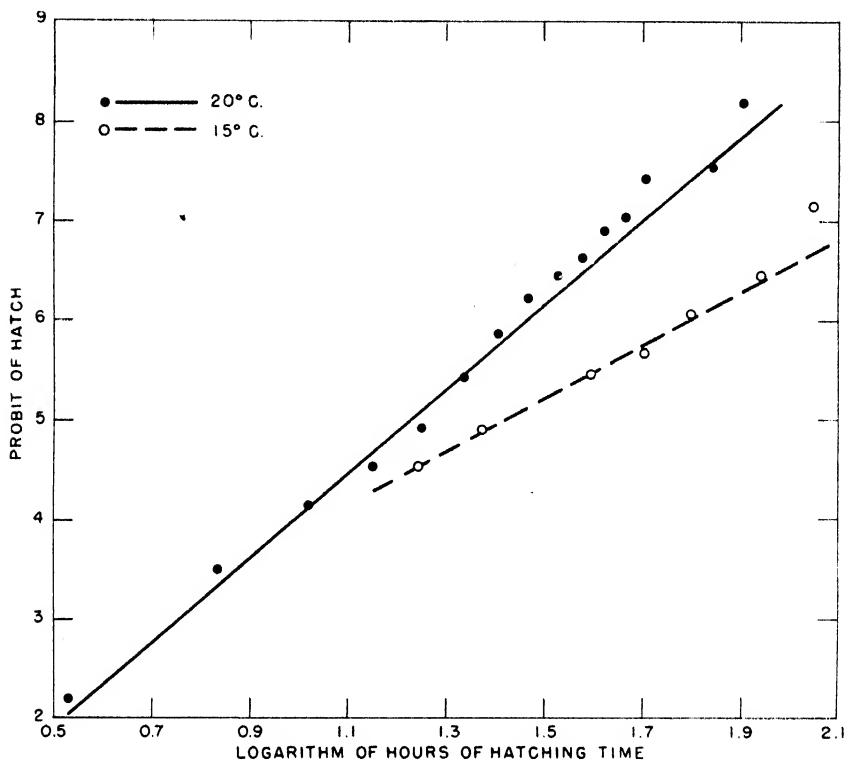


FIGURE 1.—Hatch of *Anastrepha serpentina* eggs at 15° and 20° C., in terms of probits and logarithms of $(T - I)$. At 15° $y = 2.67 \log (T - 361.0) + 1.21$. At 20° $y = 4.21 \log (T - 144.0) - 0.16$.

The converted hatching data for each test summarized in table 1 were plotted, and the results obtained at 15° and 20° C. are shown in figure 1. In each case approximately a straight line was indicated, which may be represented by the general equation

$$y = m [\log (T - I)] + b \quad (1)$$

where y is the probit of the percent cumulative hatch, m is the slope of the line or the rate of hatch, T is the developmental time for the portion of the eggs represented by y , I is the incubation period, and b is a constant which corrects for the fact that the line may not pass through the origin.

The incubation period as determined in these studies is not completely independent of the number of eggs that hatch. A large sample has a broader hatching range than a small one, the first egg hatching

⁷ BLISS, C. I. THE RELATION BETWEEN EXPOSURE TIME, CONCENTRATION AND TOXICITY IN EXPERIMENTS ON INSECTICIDES. Ent. Soc. Amer. Ann. 33: 721-766, illus. 1940

a little sooner and the last egg considerably later. For a given temperature the observed incubation period should be constant within about 4 percent, which is sufficiently accurate to be used as a "time constant" in these calculations. The incubation period could be determined more exactly by a combination of more frequent observations and larger numbers of viable eggs. The true value could be expected with an infinite number of viable eggs and continuous observation.

A study of the variability of the incubation period with the number of viable eggs was based on the equation for the line for 20° C. in figure 1. The incubation period of 144 hours (table 1) was found experimentally with 387 viable eggs and observations approximately every 3 hours. Assuming hourly observations and 144 hours as the true incubation period, the relation between the number of viable eggs and the observed incubation period was calculated. The expected hatch at any time for any given number of viable eggs may be calculated from the equation. When any value below 50 is selected for the percent hatch, the first egg to hatch automatically fixes the total number of viable eggs. The percent hatch as used above is not the percent of viable eggs, but the percent of the total viable eggs that have hatched at a given time. For example, at 147 hours a probit of 1.85 is calculated from the equation at 20°. The probit is equivalent to 0.082 percent. In order for the first hatched egg to be 0.082 percent hatch, there must be 1,220 viable eggs. From a group containing 1,220 viable eggs hatch should be observed at 147 hours, but not at 146; therefore, the incubation period is recorded as 146 hours in table 2.

TABLE 2.—Expected variation in the estimation of the incubation period with the number of viable eggs of *Anastrepha serpentina* at 20° C.

Number of viable eggs	Incubation period	Number of viable eggs	Incubation period
	Hours		Hours
20,000	145	76	148
1,220	146	33	149
233	147	18	150

If as many as 233 viable eggs were used in an experiment, and observations were taken hourly before the initial hatch, the difference in incubation period would be 2.1 percent. Even with as few as 18 viable eggs the error would be only 4.2 percent.

The two experiments which were run at 25° C. gave values of the incubation period in agreement with the theory. With 79 and 276 viable eggs the observed incubation periods were 90 and 86.8 hours, respectively. The difference was less than 4 percent.

The reaction of the first individual, a method used successfully by Baker and coworkers,⁸ gives a useful measure of the incubation period, because a relatively large portion of the eggs hatch during the first part of the hatching period. The distribution becomes normal when the time units are converted to logarithms, and the reaction of the first individual is stabilized by the large percentage of the population which reacts within a short time after the first.

⁸ See footnote 3, p. 266.

The effect of temperature on the hatching process may be conveniently summarized by showing the relationship of temperature to each of the constants given in equation 1. As shown in table 3, between 15° and 30° C. the incubation period (I) is inversely proportional and the rate of hatch (m) directly proportional to the temperature. The value of b is variable, but this variation is in no way correlated with the temperature. Both the incubation period and the rate of hatch indicate that 30° is close to the optimum temperature. The rate of hatch was decreased at 32.5°, whereas the incubation period was nearly the same at 30° and 32.5°.

TABLE 3.—Observed and calculated values for incubation period and rate of hatch of *Anastrepha serpentina* eggs at different temperatures

Temperature (t), ° C.	Incubation period (I)		Rate of hatch (m)		Constant (b)
	Observed	Calculated	Observed	Calculated	
	<i>Hours</i>	<i>Hours</i>			
15	361.0	360.5	2.67	2.89	1.21
20	144.0	142.7	4.21	3.78	— .16
25 (a)	90.0	88.9	4.43	4.66	.68
25 (b)	86.8	88.9	4.70	4.66	.73
30	64.9	64.6	5.53	5.55	— .08
32.5	64.5		4.40		1.32
Average					.62

The equations representing the effect of temperature on these two constants were obtained by the method of least squares.

$$1/I = 0.0008471t - 0.009933 \quad (2)$$

$$m = 0.1768t + 0.242 \quad (3)$$

The observed and calculated values given in table 3 indicate that the equation is fairly exact for the incubation period. The graphic presentation of the temperature effects is shown in figure 2. It will be seen that any deviation from the linear temperature-velocity relationship in the hatching process must occur above 30° and below 15° C.

By means of equations 2 and 3 it is possible to calculate the incubation period and rate of hatch at any temperature between 15° and 30° C. within the limits of error. With these calculated constants hatching curves were calculated at each of the temperatures studied. The agreement between the calculated curves and the actual observations, which is shown graphically in figure 3, is usually within 5 hours. Since no relationship was developed for the percentage of hatch as affected by the temperature, the number of viable eggs as found by experiment was taken for the calculations.

LARVAL STAGE

METHODS USED IN STUDY

Following a technique devised by W. E. Stone (unpublished), newly hatched larvae were removed from incubation dishes with a camel's-hair brush and placed individually in a notch cut into the edge of a thin slice of fruit. The slice of fruit was sandwiched between two pieces of glass (2½ by 2¾ inches) and sealed in place by dipping the edges of the glass mount into melted paraffin (fig. 4). Excess juice

was removed from the slice of fruit to avoid drowning the larva. A few holes pushed through the paraffin between the edges of the mount provided aeration. Since fruit held between glass slides disintegrated

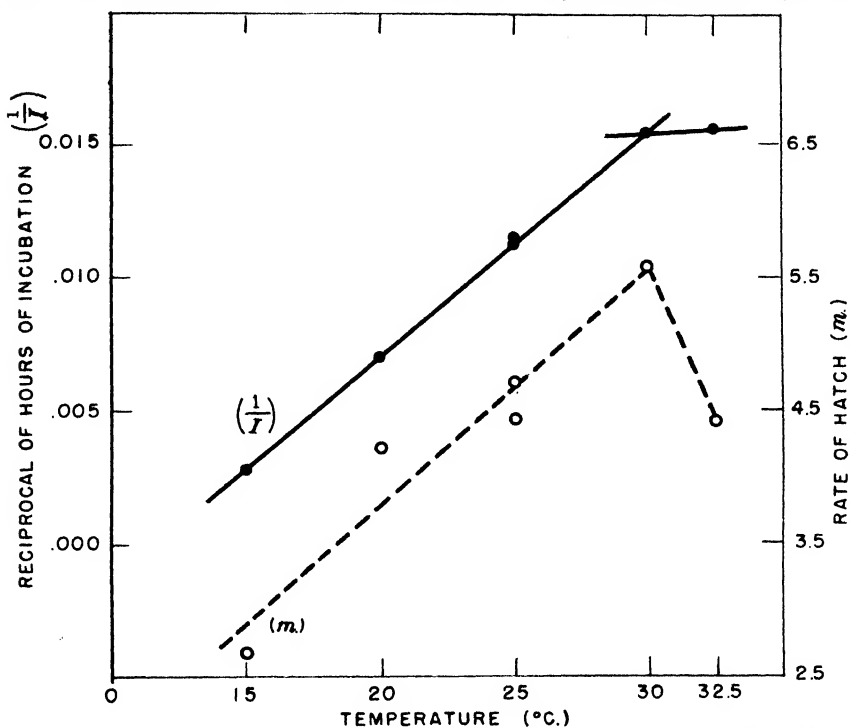


FIGURE 2.—Effect of temperature upon the incubation period and rate of hatch of *Anastrepha serpentina* eggs.

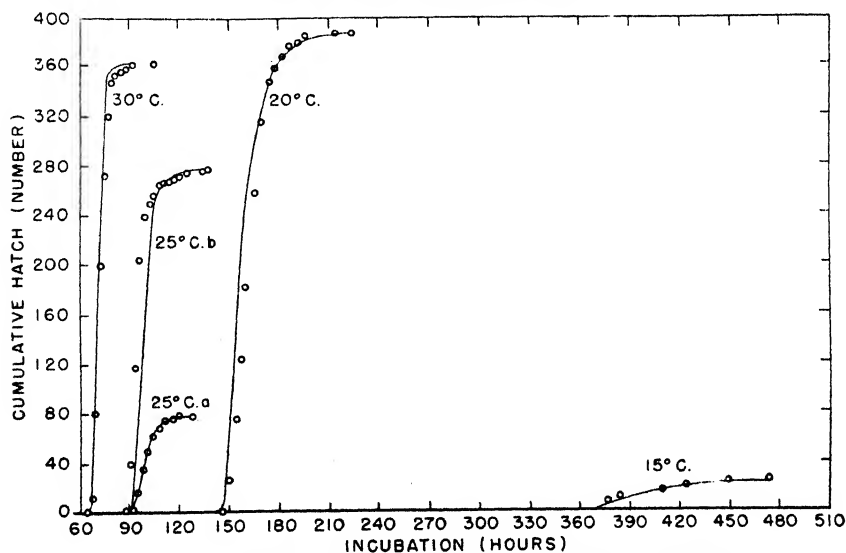


FIGURE 3.—Egg-hatching curves for *Anastrepha serpentina*.

at the higher temperatures, it was necessary to transfer larvae to fresh slices about every 24 hours. Fungus growths on fruit were reduced to a minimum through frequent changes of the media and by the use of sterilized instruments and glass plates.

The early studies on larval development were made with a white-fleshed variety of peach. Later in the season it became necessary to substitute a yellow-fleshed variety. The latter proved to be much superior, because the flesh did not darken for at least 24 hours and the cast skins and mouth hooks of larvae were readily visible against such a background.

Chicozapote when mealy ripe furnished a more satisfactory medium for rearing the larvae, but the mottled coloration of fruit cells made it difficult to locate first instars. When the fruit reached this stage, it

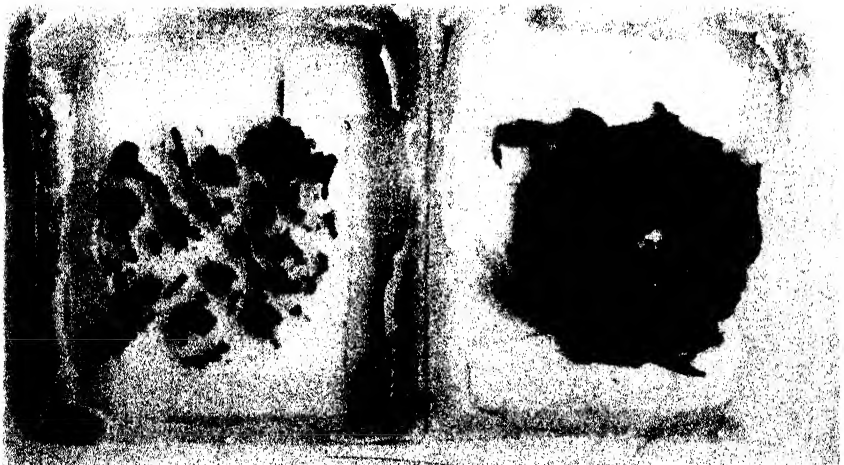
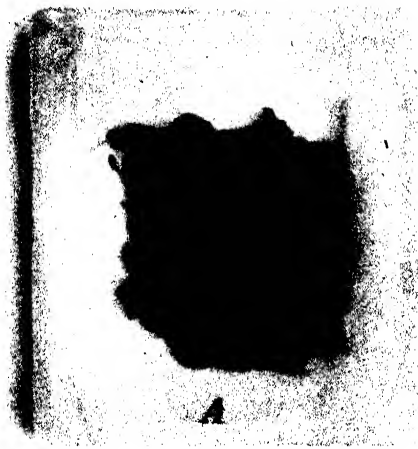


FIGURE 4.—Mount with slice of chicozapote held between glass plates, showing larva and larval tunnels of *Anastrepha serpentina*: A, Closed tunnels; B, open tunnels.

was held in a refrigerator to avoid rapid desiccation. Green fruit and fruit allowed to ripen in cold storage apparently possessed certain qualities that inhibited larval development, for in early studies at 25° C. only 1 out of 50 larvae survived when placed in green chicozapote. Later, when ripe chicozapote was used, 11 out of 15 larvae mounted were reared through the pupal stage at this temperature.

Larvae were held in a succession of sandwich mounts until late in the third stadium, when they usually became sluggish. Each larva was then placed in an excavation in a freshly cut piece of fruit, which was held over sterilized sand in a sterilized crystallization dish. The larva could thus feed on the fruit or enter the sand to pupate. Constant vigilance was required to prevent the growth of fungi. The fruit was changed often, and mycelial threads found on puparia were removed with a fine brush moistened with dilute alcohol and cupric chloride solution. The fruit was removed from the dishes as soon as the larvae pupated. Puparia were examined daily, and the sand was kept moist with a 0.1 percent cupric chloride solution.

MOLTING PROCESS

This fruitfly, in common with many other Diptera, has three larval instars. Larvae in process of molting were seen on several occasions. In one instance a first instar held at 25° C. was observed changing to a second instar. When first noticed the larva was lying on its side in a "semiparalyzed" condition and possessed mouth hooks of both instars (fig. 5). The first-instar hooks were in front and to one side of the new set of hooks, which, of course, were an integral part of the larva. The larva made vigorous efforts to free itself of the mouth hooks and skin. For about 20 minutes it kept rolling over and making frequent weak jabs with its head, occasionally contracting lengthwise and bending in such a way as to jab the end of its body. During the next 15 minutes its activity increased, the larva making numerous longitudinal propulsions posteriorly, until the cast skin was ruptured or loosened and the molting was completed. The larva then crawled away and within 4 or 5 minutes began feeding voraciously.

LENGTH OF LARVAL PERIOD

Except for 12.5° C., the larval development was studied at the same temperatures that were used in the study of the egg stage. The tests at 10° C. comprised 61 larvae that emerged from eggs incubated at 25° which were mounted in peach, and 15 larvae from eggs incubated at 20° which were mounted in chicozapote. No larvae held at this temperature attained the second instar. At 15° 1 out of 30 larvae placed in peach completed the larval stage, as compared with 4 out of 11 larvae in chicozapote.

Temperatures at 20°, 25°, and 30° C. are considered the optimum for larval survival (table 4). The length of the larval period, especially the third stadium, varied widely at 25° and 30°. Much of this variation is ascribed to differences in time spent in a semidorimant condition before pupation. Some third instars that appeared to be full grown continued to be very active although they did not feed. For this reason it was not always possible to determine with certainty just when larvae should be transferred from fruit held between glass plates to fruit held over sand.

The single larva to develop at 32.5° C. spent 2.5 days in the first stadium and 1.5 days in the second. At 35° from 4 to 9 days were

spent in the first stadium and 5 to 8 days in the second, with death ensuing in the third. It appears, therefore, that the delay in development was due to the high temperature.

Of 77 larvae mounted in slices of peach at 37.8° C., none survived long enough to reach the second instar. On the other hand, the thin

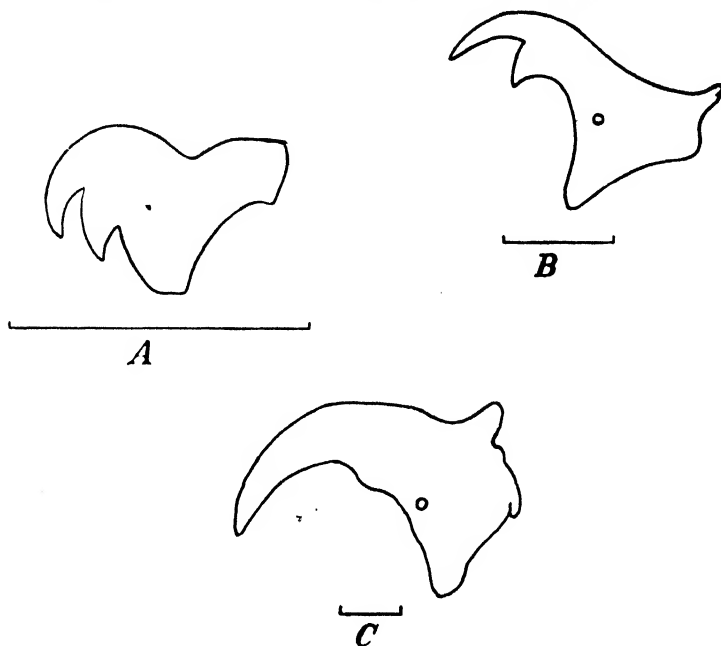


FIGURE 5.—Mouth hooks of larvae of *Anastrepha serpentina*: A, First instar; B, second instar; C, third instar. The line below each figure represents 0.04 mm.

TABLE 4.—Average length of immature stages of *Anastrepha serpentina* when held at various temperatures on peach and chicozapote
DEVELOPMENT ON PEACH

Temperature (°C.)	Individuals	Egg stage	Larval period				Pupa- rial stage	Total, egg to adult	Sex of adults ¹	
			First	Second stadium	Third stadium	Total			Male	Female
	Number	Days	Days	Days	Days	Days	Days	Days	Number	Number
15	1	² 16	12	17	21	50	55	121	0	1
20	2	7	7.5	4	12.5	24	26.5	57.5	1	1
25	15	4.2	3.8	3.4	9.1	19.1	16.5	39.8	6	8
30	7	3.1	3.7	3.3	10.4	17.4	12.4	33.0	2	5
DEVELOPMENT ON CHICOZAPOTE										
15	4	² 16	12.3	9	20.3	41.5	³ 56.8	114.3	4	0
20	19	7	4.9	3.5	11.1	19.4	27.3	53.7	4	15
25	12	4.3	3.5	2.8	8.9	15.2	16.2	35.7	5	7
30	14	3	3.1	2.4	6.2	11.7	12.3	26.9	7	7
32.5	1	⁴ 3	2.5	1.5	7	11	⁵ 12	26	0	⁶ 1

¹ At 25° C., only head of fly emerged from 1 puparium; sex not determined. At 30°, 3 of the 7 flies emerged with drooped wings, with somewhat malformed bodies, or with drooped wings and malformed bodies.

² These flies were actually incubated at 20° C.

³ In 1940 E. W. Baker obtained the following results: From approximately 100 puparia held at 15° C., 54 flies developed in 56.4 days, and from the same number held at 32.5°, 2 flies developed in 12 days.

⁴ This fly was actually incubated at 25° C.

⁵ Fly was small, with drooped wings.

slices of chicozapote dried rapidly at this temperature and did not supply the larvae with suitable food. The developmental period was invariably longer when larvae were reared in peach than when reared in chicozapote.

PUPARIAL STAGE

All larvae able to complete development were reserved for observation in the puparial stage. The length of time spent in this stage is summarized in table 4. The fruit on which the larvae were reared had no effect on the length of the puparial stage. A comparison indicated that there was no difference in duration within puparia held over sand or filter paper.

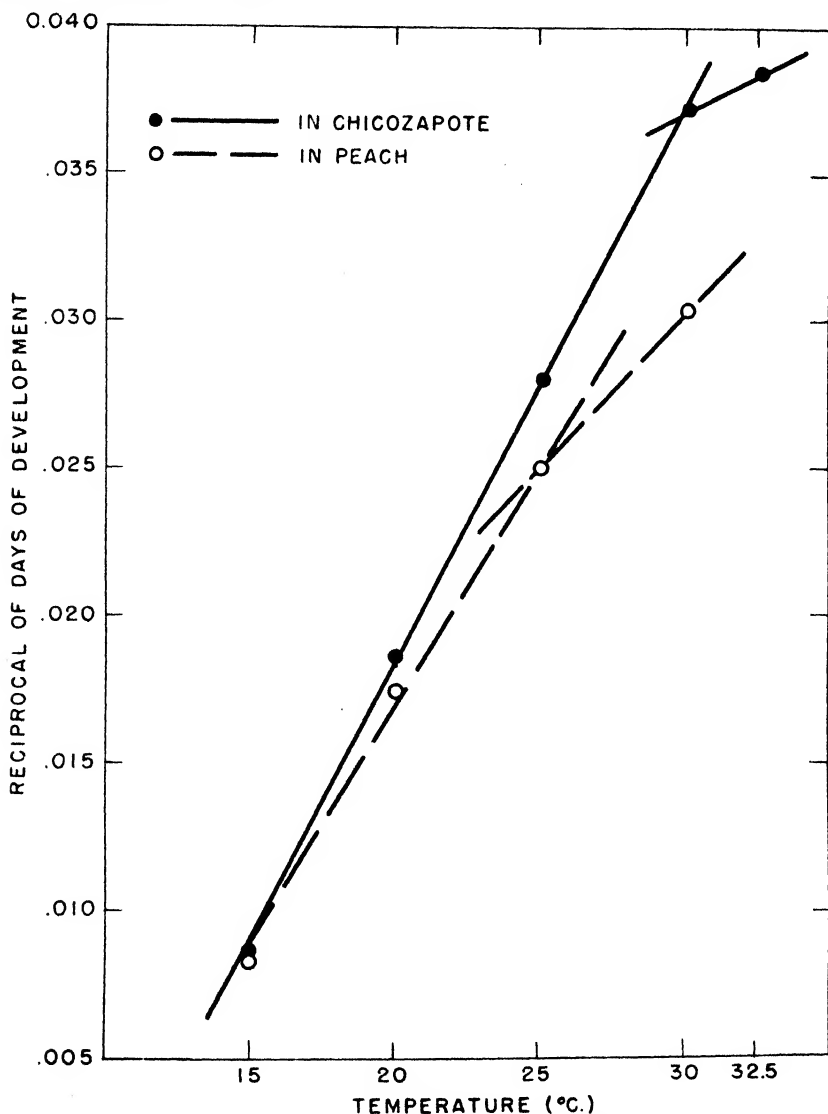


FIGURE 6.—Development of *Anastrepha serpentina* from egg to adult.

TOTAL LENGTH OF DEVELOPMENTAL PERIOD

The total length of the developmental period, egg to adult, was longer in peach than in chicozapote (table 4), and a much smaller percentage of individuals were found to complete development. When the rate of development, measured by the reciprocal of time in days, was plotted against temperature (fig. 6), linear regression lines were produced which showed the contrast in the rate of development in the two hosts. Data on the egg and larval stages indicate that the lower limit of development is between 10° and 15° C. and the upper limit between 32.5° and 35°, but somewhat lower temperatures are probably required for normal fly emergence. Flies with drooped wings, with malformed bodies, or with drooped wings and malformed bodies were observed at 30° and 32.5°. Again chicozapote appeared to be a better medium than peach.

SUMMARY

As *Anastrepha serpentina* (Wied.) is known to develop in several fruits of economic importance, the development of the immature stages of this fly was studied.

First oviposition occurred 16 days after flies began to emerge from puparia. Eggs were procured from confined flies by exposing arcs of fruit skins fastened by paraffin to glass plates. The fact that this fruitfly preferred chicozapote skins to peach skins for oviposition was shown to be highly significant.

Eggs for incubation and hatching studies were held in Syracuse watch glasses in cabinets at temperatures ranging from 10° to 37.8° C. Hatch occurred from 15° to 32.5°, and the incubation period ranged from 361 to 64.5 hours.

After the incubation period the hatching process approximated a straight line when the probit of the cumulative percent hatch was plotted against the logarithm of time. The temperature effect on the reciprocal of the incubation period and on the rate of hatch was measured so that approximate hatching curves might be calculated at any temperature from 15° to 30°.

The larvae were reared at temperatures from 15° to 32.5° C. by placing them in slices of fruit held between glass plates. Mealy-ripe chicozapote provided the better medium for larval development but peach was also used.

Molting of the first instar was observed in some detail. The second stadium was generally shorter than the first, but the third was considerably longer than either of the others.

In peach larvae developed in 50 to 17.4 days between 15° and 30° C., whereas in chicozapote development required only 41.5 to 11.7 days in the same temperature range and 11 days at 32.5°.

Holding and caring for puparia in crystallization dishes is described. The developmental time was nearly the same for puparia formed from larvae fed on peach and on chicozapote.

The total development, egg to adult, ranged from 121 days for one individual reared in peach at 15° C. to 26 days for individuals reared in chicozapote at 30° and 32.5°. Development in peach was invariably longer than in chicozapote, the difference being in the feeding or larval stage.

When the rate of development in each fruit, measured by the reciprocal of time in days, was plotted against temperature, linear regression lines were produced.

STUDIES IN THE CHEMISTRY OF GRASS SILAGE¹

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INTRODUCTION

In connection with an attempt to find substitutes for molasses as preservatives for silage in our wartime economy, and to ascertain whether good quality grass silage could possibly be made without any preservative, a rather extensive study of the biochemistry of numerous kinds and types of silage was carried on during the seasons 1942-43 and 1943-44.

The literature of silage making is voluminous and no good purpose would be served by attempting to review all or even any considerable part of it here. The reader is referred to reviews by Bender and Bosshardt (2),² Hopkins and Ripley (5), LeClere (6), and Woodward and Shepherd (18). Earlier chemical studies are confined pretty largely to routine fodder analyses which, while they furnish information on feeding value, shed little light on the fermentation process or on those end products (especially volatile acids and ammonia) which determine the quality and palatability of the silage. Noteworthy exceptions to this statement are the work of Dox and Neidig (3, 4), Neidig (9), and Swanson and Tague (15), although Dox and Neidig worked only with the corn plant. Recent contributions on the subject which are of significance here include the work of Watson and Ferguson (17), Perkins (11, 12), and Stone et al. (14).

EXPERIMENTAL METHODS AND MATERIAL

Forty samples of silage representing various crops and treatments were analyzed to determine moisture content, pH values, total nitrogen, water-soluble nitrogen, carotene, volatile bases, total acidity, amino acids, lactic acid, acetic acid (total and free), and butyric acid (total and free). In addition, the first five of the above entities were determined in nine samples of the fresh green crops before ensiling. The methods used were: For moisture, the Brown-Duvel toluene distillation procedure as modified by the Association of Official Agricultural Chemists (1, p. 353); pH values, a water extract of the finely chopped silage, using a Coleman pH meter with glass electrode; total nitrogen in the fresh sample by the usual Kjeldahl procedure; water-soluble nitrogen, the method of Watson and Ferguson (17); carotene, the method of Wall and Kelley (16); volatile bases, total bases, total

¹ Received for publication October 12, 1944. Contribution No. 538 of the Massachusetts Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 287.

acidity, and amino acids by the method of Foreman as outlined by Watson and Ferguson (17); acetic and butyric acids by the method of Wigner as given by Watson and Ferguson (17); and lactic acid by difference as suggested also by these workers, i. e., total acidity—(amino acids+acetic acid+butyric acid)=lactic acid. In addition to the above the customary fodder analyses were also made.

In sampling from the large silos on the college farm small handfuls of silage were taken here and there over the entire area of a freshly exposed surface, sufficient to fill a friction-covered tin pail of 6-quart capacity. Samples submitted from farms were usually of much greater volume (a bushel or more), thus affording an opportunity for careful subsampling. Samples from the miniature silos were taken from all through that part of the mass of silage which was not obviously spoiled by rot or mold. All determinations except fat, fiber, and ash were made on the fresh samples finely chopped with scissors just before weighing. Total nitrogen was determined in both fresh and dry material.

The samples may be divided into 3 lots: (1) A series of 20 samples (16 of silage and 4 of fresh unensiled crops) which were obtained in a study of 2 crops treated with various preservatives in miniature silos, during the season of 1942-43; (2) a series of 19 samples of different origin and nature, obtained also in 1942-43, 13 of which were sent in by cooperating farmers³ interested in the project, the other 6 being obtained from the large silos on the college farm; and (3) a series of 10 samples (5 of silage and 5 of the corresponding crops before ensiling) obtained from 1 of the large silos on the college farm during the season of 1943-44.

As already noted, the first lot of samples came from miniature silos. The objective of the study with these was to ascertain whether results might be obtained from small containers which would be similar to those secured from large silos. Statements in the literature are conflicting on this point; it is obvious that if small containers could be used with confidence in the results obtained, progress toward the main objectives of the investigation would be greatly accelerated.

The containers employed for the purpose were heavy glass cylinders, 9.5 inches inside diameter, and 39 inches high, open at both ends. The volume was slightly under 1.5 bushels. Sixteen such containers were used in accordance with the schedule in table 1. They were subjected to as much pressure as was practicable (120 pounds per square foot) and were kept in a darkened room.

The second lot of samples was obtained for the most part without resort to any special experimental set-up. All were from large silos (100 tons capacity or more) and represented conditions regularly existent in practical operations. Two of them can be compared with certain samples in the previous group; they were from the same lot of mixed grass ensiled at the same time, the only difference being that they were from 100-ton silos on the college farm. Thus a direct comparison was provided between large silo and small container on the same lot of grass and with the same preservative.

³ Acknowledgment is made of the cooperation of these men and more especially, of the interest taken by Herbert A. Brown, county agricultural agent at Concord, Mass., who was instrumental in obtaining the samples.

TABLE 1.—General outline and sample identification ¹

LOT 1 (FROM MINIATURE SILOS)	
Mixed grasses: ²	8. Lactic acid culture and salt.
1. The fresh, unensiled crop; Nos. 5, 6, 7, and 8 represent the ensiled product.	9. As in No. 5 plus a little water.
2. As in 1; Nos. 9, 10, 11, and 12 represent the ensiled product.	10. As in No. 6 plus a little water.
Alfalfa: ³	11. As in No. 7 plus a little water.
3. Unwilted. The fresh crop; Nos. 13, 14, 15, 16, and 17 represent the ensiled product.	12. As in No. 8 plus a little water.
4. Wilted for 27 hours; Nos. 18, 19, and 20 represent the ensiled product.	Alfalfa silage:
Mixed grass silage:	13. Unwilted. No preservative.
5. No preservative.	14. Unwilted. Lactic acid culture.
6. Lactic acid culture—66 lbs. (<i>Lactobacillus bulgaricus</i>).	15. Unwilted. Molasses—90 lbs.
7. Salt—18 lbs.	16. Unwilted. Salt.
	17. Unwilted. Lactic acid culture and salt.
	18. Wilted. No preservative.
	19. Wilted. Lactic acid culture.
	20. Wilted. Molasses.
LOT 2 (MISCELLANEOUS SAMPLES—ALL SILAGES)	
Mixed grasses:	Oats:
21. No preservative. Similar to 5 and 9, except from a large silo.	30. No preservative.
22. Lactic acid culture. Similar to 6 and 10, except from a large silo.	31. Lactic acid culture and salt (16 lbs.)
23. Lactic acid culture and salt—15 lbs.	Oats and peas:
24. Molasses—40 lbs.	32. No preservative) From the same silo 7 weeks
Mixed grass and clover:	33. Do.) apart.
25. Lactic acid culture and salt—8 lbs.	34. Salt—10 lbs.
26. Molasses—50 lbs.	Winter wheat:
Timothy and clover:	35. Salt—a pailful to a load.
27. No preservative.	Soybeans:
28. Molasses—amount not known.	36. Molasses—amount not known.
29. Molasses—35 lbs.	Sudan grass, millet, and sorghum:
	37. Molasses—amount not known.
	Corn:
	38. No preservative }
	39. Do. } Included for comparison.
LOT 3 (FROM LARGE EXPERIMENTAL SILO)	
Mixed grass and clover:	45. Molasses (75 lbs.) and urea (10 lbs.). No. 40 after ensiling.
40. The freshly chopped crop after preservative has been added, but before ensiling.	46. Urea (10 lbs.) No. 41 after ensiling.
41. Do.	47. Ground wheat—100 lbs.—No. 42 after ensiling.
42. Do.	48. Ground wheat—150 lbs.—No. 43 after ensiling.
43. Do.	49. Ground wheat—200 lbs.—No. 44 after ensiling.
44. Do.	

¹ Amounts of preservative given are per ton of green crop; where not stated they are the same as previously noted in the lot in question. In the large silos lactic acid culture was applied at several levels, being first diluted with water; a sprinkling can full of the diluted culture was used at each level.

² Healed to some extent but not in bloom.

³ In full bloom.

The third lot was from a definite experimental set-up in a 100-ton silo; five different lots of silage were treated as outlined in table 1. Assurance that each sample of silage would represent material identical, or nearly so, with that of the corresponding sample of fresh crop taken at filling time was provided for by the following procedure: A sample of the freshly chopped crop to which the specific preservative had been added in routine fashion was taken from the silo about mid-way of each lot; at the same time a clean burlap grain sack was filled with the same material as the sample, and, properly tagged, was buried in the center of the silo. From each sack when reached as the several lots were fed, the corresponding silage sample was secured. Table 1 outlines the scope of the work and serves to identify the several lots and samples.

CHEMICAL ANALYSES

Limitations of space preclude presentation here of the large accumulation of detailed data. Instead, general summaries and emphasis on high lights and on significant differences are depended upon to tell the story. It may be of interest to note in passing that the work involved over 800 chemical determinations (not counting parallels) in addition to several hundred other observations most of which were organoleptic. All values except moisture, pH, and carotene are reported as percentages in the dry substance; the first two exceptions are of course the values for the fresh silage; the carotene values are expressed as milligrams per pound of dry silage. Not all of the various entities determined are reported; it became evident upon critical examination that some of them showed only minor variations from sample to sample, and hence contributed nothing of significance to the findings. Table 2 summarizes the results from two standpoints—variation due to (1) kind of crop ensiled, and (2) kind of preservative used.

TABLE 2.—Composition of silage as influenced by kind of crop and by preservative used ¹

Crop ensiled and preservative used	pH	Volatile bases ²	Total acidity ³	Lactic acid	Acetic acid	Butyric acid	Carotene
		Percent	Percent	Percent	Percent	Percent	Mg. per pound
Crop:							
Alfalfa	4.5	0.76	14.41	4.75	5.49	0.66	46
Mixed grasses	5.2	1.49	11.43	1.16	1.84	7.29	80
Mixed grasses and clover	4.6	.76	9.63	3.64	1.50	2.98	68
Timothy and clover	4.5	.78	12.13	3.26	3.52	2.80	77
Oats	5.2	.60	7.65	2.06	1.65	2.40	33
Oats and peas	4.5	.46	10.96	3.96	2.66	.50	39
Winter wheat	5.4	.81	11.28	None	2.26	6.83	117
Sudan grass, millet, and sorghum	4.5	.58	9.66	1.13	2.28	4.42	13
Soybeans	4.3	.54	13.00	4.73	4.42	1.64	28
Corn (included for comparison)	3.8	.26	14.64	7.58	5.22	None	27
Preservative:							
None ⁴	4.8	.92	11.26	2.58	2.69	2.87	58
Lactic acid culture	4.7	1.14	12.89	2.62	3.27	4.21	63
Salt	5.0	1.08	11.61	1.04	2.88	5.13	77
Lactic acid culture and salt	5.3	1.14	11.23	.19	3.34	5.38	63
Molasses	4.5	.74	12.48	4.26	3.21	3.04	62
Molasses and urea	5.2	1.70	10.63	4.16	1.92	2.74	63
Urea	5.6	3.80	13.11	None	5.68	6.44	130
Ground wheat	4.3	.50	9.02	5.06	1.04	2.49	54

¹ The values in this table are composites, and include all samples listed under the specific corresponding category in table 1.

² Volatile bases are expressed as percentage of ammonia in the dry substance.

³ Total acidity is expressed as the weighted percentage of amino, lactic, acetic, and butyric acids in the dry substance of each sample, based on the molecular weights of the acids in question. (Amino acids were calculated as an average of aspartic and glutamic acids, based on their molecular weights.)

⁴ Does not include the values for corn silage.

Variations due to the crop used were more significant than those due to the action of preservatives. The principal points of interest are:

(1) In general the legume silages developed a higher acidity than those made from grasses or small cereals; also the presence of a legume in a mixture resulted in higher acidity. The inverse relationship held true for volatile bases, which is what would be expected.

(2) For the most part also the legumes developed a higher total acidity. Differences, however, are more evident in the type of acidity than in the total amount. The legume silages were characterized by a relatively high content of lactic and acetic acid and their influence

in this respect is also evident in the mixed crop silages; the grass silages and certain of the small cereals, on the other hand, had a relatively high butyric acid content and little or no lactic acid.

(3) The two lots of corn silage offer a striking contrast to the grass silages in their low pH, values very low volatile base content, high lactic and acetic acid, and absence of butyric acid. This seems an appropriate point at which to make the general observation that the most strongly acid-smelling silages have frequently been the least acid, as judged either by pH or by total acidity as determined by titration. This may seem paradoxical until one considers that the type of fermentation which develops butyric acid is likely also to develop more ammonia and other bases which neutralize a portion of the acid and keep the pH relatively high and the titration values low. The better quality silages with mild, pleasant odor were almost invariably more acid than those which sense of smell alone would rank as much more acid. The acid in the better quality silages was largely lactic, which is non-volatile and of relatively mild odor, and their volatile base content was usually quite low.

(4) Of the preservatives used molasses and ground wheat seem to have been the only ones that were at all effective. They kept the break-down of protein at a relatively low level, as evidenced by low content of volatile bases; favored the formation of lactic acid; and limited the formation of butyric acid. Salt and lactic acid cultures were in many respects worse than no preservative at all; they encouraged the formation of volatile bases and butyric acid and inhibited the development of lactic acid, some of the samples showing none at all. Urea was evidently entirely unsuited to the purpose; the idea in using it was to increase the nitrogen content of the silage and hence the potential protein level for ruminants. It seems apparent from the high pH and the very high content of volatile bases in this lot of silage that a considerable portion of the urea must have been converted into ammonia. That some of it was lost by leaching is evident from the increased nitrogen content of the leachings from this particular silo. The average figure for 4 years previously was 0.30 percent nitrogen; when urea was used it rose to 0.56 percent. The silage made with urea alone as a preservative had a very objectionable odor and was unpalatable to milking cows. The silage made by using ground wheat as a preservative had a mild, pleasing odor and was very palatable. The herdsman in charge observed that it was the best quality grass silage he had fed in our 8 years of experience. In table 3 is presented a summary of the proximate analyses,⁴ together with some interesting contrasts between certain of the silages and the fresh crops from which they originated.

Changes in composition due to the silage fermentation show a definite trend regardless of the nature of the crop ensiled. Relatively the greatest losses are in carotene and the greatest gains are in ether extract. The gains in ash and fiber doubtless are apparent only, because of losses of a similar magnitude in protein and nitrogen-free extract. The gain in ether extract is actual, since the organic acids formed by fermentation from the carbohydrates are ether-soluble,

⁴ Acknowledgment is made of the services of Albert F. Spelman, assistant chemist, and Leo V. Crowley and C. Tyson Smith, junior chemists, in the Feed Control Laboratory of this station, who made the fodder analyses under the direction of John W. Kuzmeski, senior chemist, and Philip H. Smith, chief chemist.

whereas carbohydrates are not soluble in anhydrous ether. High carotene values did not necessarily occur in silages that were of good quality in other respects; e. g., note the very high value for the winter wheat silage. In most other respects this was about the poorest quality silage examined; a similar condition was noted in a lot of mixed grass and clover preserved with urea (see table 2). Stage of maturity of the crop probably had a good deal to do with this. In the case of the silage preserved with urea it may be that the type of fermentation induced by the high pH altered certain noncarotene pigments sufficiently to permit their estimation as carotene.

TABLE 3.—Average proximate composition of silages from various crops and crop combinations, and of some fresh, unensiled crops from which certain of the silages were prepared ¹

Kind of crop	Moisture as sampled or received	pH	Carotene	Percentages in the dry matter				
				Total ash	Crude protein	Crude fiber	N-free extract	Ether extract
	<i>Percent</i>		<i>Mg. per pound</i>					
Alfalfa	62.3	6.0	133	7.3	17.8	35.7	37.4	1.8
Alfalfa silage	68.2	4.5	46	9.3	16.8	38.5	32.8	2.6
Grasses, mixed	75.0	6.1	157	7.3	12.8	33.9	43.3	2.7
Grasses, mixed, silage	74.8	5.2	80	² 10.2	10.0	38.7	38.0	3.1
Grasses and clover	68.1	6.2	58	8.0	12.1	32.8	44.9	2.2
Grass and clover silage	68.9	4.6	68	7.8	10.5	37.2	41.0	3.5
Timothy and clover silage	73.8	4.5	77	10.6	12.9	34.7	38.8	3.0
Oat silage	70.6	5.2	33	9.2	9.1	38.0	40.3	3.4
Oat and pea silage	67.3	4.5	39	8.7	10.4	35.8	41.7	3.4
Winter wheat silage	73.9	5.4	117	12.6	8.0	41.4	34.5	3.5
Sudan grass, millet, and sorghum silage	79.7	4.5	13	7.9	4.6	44.5	40.9	2.1
Soybean silage	71.0	4.3	28	19.7	10.9	30.4	37.2	1.8
Corn silage (for comparison)	74.2	3.8	27	6.0	8.4	35.3	47.6	2.7

¹ The values in this table are composites, and include all samples listed under the specific corresponding category in table 1.

² This figure is due in part to the use of salt in some of the silages.

Table 4 shows the composition of material ensiled in the miniature silos referred to earlier in comparison with material from the same lot of grass ensiled with the same treatment in two 100-ton silos. Except for carotene the differences are rather small; what there are indicate that fermentation took place on a more extensive scale in the large silos. The differences in carotene content are arresting; if typical of what may occur in large silos they represent a need for further investigation in order that such excessive losses of this important nutrient may be prevented. These losses are on a par with those occurring in dry hay. It should be mentioned that the small containers did not prove satisfactory. Spoilage losses were high in most of the containers, averaging over 18 percent, due chiefly to rotting of the upper layers. In the containers in which wilted alfalfa was stored the losses were excessive, averaging over 60 percent, due to penetration of mold. Fermentation losses were normal in most cases, averaging about 9 percent with some under 5 percent. Evidently conditions existent in a large silo are not readily duplicated on a small scale.

TABLE 4.—Composition of silage from the same lot of grass ensiled in small silos (1.5 bushel capacity) and large silos (100 tons capacity)

Type of silo and treatment of silage	Moisture when sampled	pH	Carotene	Percentages in the dry matter									
				Volatile base	Total acidity	Lactic acid	Acetic acid	Butyric acid	Total ash	Crude protein	Crude fiber	N-free extract	Ether extract
	Pct.		Mg. per pound										
Small silos—no preservative	78.3	5.1	107	1.74	12.15	None	1.92	7.89	7.6	10.2	38.0	40.6	3.
Large silos—no preservative	73.4	5.4	25	1.59	13.22	None	.80	8.08	8.7	9.2	40.4	38.2	3.
Small silos—lactic acid culture	76.4	5.0	84	1.44	10.91	None	2.15	7.55	8.9	10.3	38.8	39.0	3.
Large silos—lactic acid culture	71.4	4.9	32	1.36	12.61	0.73	2.46	5.70	9.8	9.3	39.8	37.9	3.

In table 5 appear the contrasts in composition due to wilting of alfalfa. Changes in composition before ensiling caused by wilting seem to have been confined to a material reduction in carotene content and to rather minor and probably only relative increases in protein and nitrogen-free extract with a corresponding reduction in fiber. Changes after ensiling are of much more significance; note especially the lactic acid-acetic acid relationship in the wilted and unwilted silage, and the much lower fiber and higher protein and nitrogen-free extract of the wilted silage. Insofar as these silages are concerned wilting resulted in a very different type of fermentation and final product. It may be that the influence of mold enters in here; the excessive development of mold in the wilted silages has been mentioned already, but it should be noted that care was taken to discard all visible signs of mold when the samples were taken for analysis.

CHLORIDE CONTENT OF SILAGE

In the course of the work the question arose as to whether the practice of using salt as a silage preservative might not at times introduce an excessive amount into the rations of livestock, and this in turn suggested the need to know how much salt (or total chlorides) occurs in silage naturally. A search of the literature showed very little information on this point, so a determination of the chloride content of these 40 silages was made a part of the routine procedure. The determination was made on suitably diluted duplicate aliquots of the water extract by titration with N/10 silver nitrate. A 10-percent solution of potassium chromate was used as indicator, the end point being determined by the first permanent color change visible against the background of a glazed white porcelain evaporating dish. The results are summarized in table 6.

The chloride values were highest in the silages made from small grains and lowest in those made from legumes. Salt increased the chloride content to a value about $2\frac{1}{2}$ times that found in the silages with which no preservative was used. Other preservatives were without substantial effect. Consideration of the effect of the added salt upon feeding practice and animal health is appropriate here.

TABLE 5.—Composition of alfalfa, wilted and unwilted, and of the corresponding lots of silage made from it

Alfalfa	Moisture when sampled	pH	Carotene	Percentages in the dry matter							
				Volatile bases	Total acidity	Lactic acid	Acetic acid	Butyric acid	Total ash	Crude protein	Crude fiber
	Per-cent		Mg. per pound								
Unwilted before ensiling	73.5	6.0	156	(1)	(1)	(1)	(1)	(1)	7.4	17.1	37.5
Wilted before ensiling	49.0	6.0	110	(1)	(1)	(1)	(1)	(1)	7.2	18.4	34.0
Unwilted after ensiling	77.7	4.6	49	0.98	15.58	2.68	8.15	0.66	9.9	15.7	41.6
Wilted after ensiling	52.5	4.2	40	.38	12.47	8.20	1.05	None	8.2	18.6	33.4

¹ Not determined.

TABLE 6.—Average chloride content of different kinds of silage

Crop ¹	Chlorides in the dry matter ²	Preservative	Chlorides in the dry matter ²
	Percent		Percent
Alfalfa	0.80	None	1.10
Mixed grasses	1.13	Lactic acid culture	1.03
Mixed grasses and clover	1.06	Salt	2.77
Timothy and clover	1.26	Lactic acid culture and salt	2.46
Oats	1.56	Molasses	1.09
Oats and peas	1.19	Molasses and urea	1.17
Sudan grass, millet, and sorghum	1.43	Ground wheat	.88
Soybeans	.21		
Corn	.54		

¹ All samples in which salt was used as a preservative have been excluded from the average values for the several crops.² To convert these values to terms of NaCl, multiply by 1.65; to convert them to an average basis of fresh silage as fed, multiply by 0.3.

A little calculation shows that from the silages preserved with salt a cow consuming 40 pounds of fresh silage daily would get 0.55 pound of salt daily from this source alone. Assuming that she also consumes 10 pounds of a grain mixture with its usual salt content of 1 percent, she would get from these two sources somewhat over 10 ounces of salt daily; there is also the unknown quantity contained in whatever other roughage she might consume (a cow eating 40 pounds of silage usually eats 10 to 12 pounds of dry hay in addition). Information in the literature as to the amount of salt a cow requires is very meager; Maynard (7, p. 435) makes the statement that "One to two ounces of salt daily should be adequate for milking cows." Although undoubtedly cows can tolerate somewhat larger amounts than this, it is extremely doubtful whether they should receive for any considerable length of time as large an amount as 10 ounces daily. Furthermore, this is based on only the average amount found in the samples to which salt had been added; the maximum amount of chlorides found was 4.14 percent in the dry matter. Figured on the same basis as above a cow's daily salt intake on this particular lot of silage would be approximately 14 ounces; undoubtedly this is excessive.

EVALUATION OF CERTAIN METHODS

In the course of the investigation there were opportunities to compare certain analytical procedures, notably those dealing with the determination of total nitrogen and with carotene.

It has been recognized for years that in general considerable losses of nitrogen occur in all types of biological material if it is dried and ground, as for routine fodder analyses, before determining total nitrogen. Yet one finds in the literature instances of recent work where this fact has been ignored. For comparative purposes total nitrogen was determined in the fresh material immediately upon its receipt at the laboratory and again after drying and grinding. Carotene was determined by the phasic separation procedure outlined in the Official Methods (1) and by the chromatographic method described by Wall and Kelley (16).⁵ The results are shown in table 7.

Considering nitrogen first, the interesting thing is the great variation in the error due to determination after drying. On the whole, it was much larger in the silages than in the unsiled crops, but the average errors for some are so small that they might almost be classed as experimental error; e. g., that for corn or for mixed grass and clover. One might be tempted to place in this class the positive error for silage preserved with ground wheat, except for the fact that it was consistent and the average represents three different lots of silage. Apparently the ground grain served to fix volatile nitrogenous bases and thus to prevent the customary loss on drying. Molasses seems to have had a similar though less marked effect.

TABLE 7.—Comparison of (a) nitrogen determination, and (b) carotene determination in green crops and in silages by 2 methods

Material	Percentage of nitrogen in the dry matter		Percentage loss due to drying	Milligrams of carotene per pound of dry matter		Result in (2) is lower by (percent)—
	Determined in the fresh material	Determined in the dried and ground sample		(1) By phasic separation	(2) By chromatography	
Crops before ensiling:						
Alfalfa.....	3.05	2.85	5.6	(1)	(1)	(1)
Mixed grasses.....	2.08	2.05	1.4	(1)	(1)	(1)
Mixed grasses and clover ²	1.91	1.80	5.8	63	58	7.9
Kinds of ensilage:						
Crop:						
Alfalfa.....	3.10	2.69	13.2	44	36	18.2
Mixed grasses.....	2.29	1.58	31.0	113	84	25.7
Mixed grasses and clover ²	1.80	1.76	2.2	103	83	19.4
Timothy and clover.....	2.25	2.07	8.0	138	93	32.6
Oats.....	1.76	1.45	17.6	32	30	6.2
Oats and peas.....	1.77	1.67	5.6	39	19	51.3
Winter wheat.....	1.97	1.27	35.5	(1)	(1)	(1)
Sudan grass, miller, and sorghum.....	1.04	.74	28.9	29	13	55.2
Soybeans.....	2.04	1.75	14.2	56	28	50.0
Corn.....	1.37	1.35	1.5	46	27	41.3
Preservative:						
None.....	2.15	1.87	13.0	71	51	28.2
Lactic acid culture.....	2.63	2.10	20.1	90	59	34.4
Salt.....	2.43	1.63	32.9	84	67	20.2
Lactic acid culture and salt.....	2.11	1.52	28.0	89	67	24.7
Molasses.....	2.37	2.14	9.7	99	70	29.3
Molasses and urea.....	2.21	1.56	29.4	62	47	24.2
Urea.....	2.99	1.45	51.5	139	130	6.5
Ground wheat.....	1.84	1.88	+2.2	68	51	25.0

¹ Comparative figures not available.

² For obvious reasons values for those samples of mixed grass and clover to which urea has been added are not included in the averages.

⁵ Material assistance rendered and valuable suggestions made by John W. Kuzmeski, senior chemist in the Feed Control Laboratory of this station, are gratefully acknowledged.

Several investigators (8, 13, 16) have shown that when carotene is determined by alkaline extraction followed by phasic separation as outlined in the official method, varying amounts of noncarotene chromogens remain in the carotene fraction and result in carotene values that are too high. Moore (8) in particular has shown that this error is much more significant in silages than in fresh green crops; the results in table 7 confirm and extend his observations. In general, phasic separation gave carotene values about one-third higher than those obtained by chromatography, although the range was wide (from 6 percent to 55 percent higher). Variation was greatest between crops; except for the rather small difference when urea was used, variability in this respect between silages treated with different preservatives was slight, the differences between the two methods being of the same general order (about 25 percent), regardless of preservative used.

SUMMARY

A biochemical study has been made of 40 samples of different kinds of silage stored with various preservatives and of 9 samples of fresh green crops before ensiling which corresponded in identity with certain of the silages.

Variations due to the kind of crop used were more significant than those due to the action of the preservative.

In general legume silages had lower pH values and higher total acidity than silages made from grasses or small grains. The type of acidity also was different; legume silages were characterized by a high lactic acid content while the others showed relatively large amounts of acetic and butyric acids.

The most strongly acid-smelling silages were frequently the least acid, as judged by either pH or total titratable acidity. An explanation of this apparent paradox is offered.

Corn silage offered a striking contrast in composition to grass silage.

Of the preservatives used, molasses and ground wheat seem to have been the only ones at all effective. Salt and lactic acid cultures were in many respects inferior to no preservative at all. Urea was apparently entirely unsuitable for the purpose.

Changes in proximate composition due to the fermentation process showed a definite trend regardless of the nature of the crop ensiled. High carotene values did not necessarily characterize silages that were of good quality in other respects. Carotene losses in some of the large silos were excessive.

Small containers utilized in an effort to speed up the work did not prove satisfactory, losses due to rotting and mold being excessive. Evidently conditions existent in a large silo are not readily duplicated on a small scale.

Silage made from wilted alfalfa was quite different in composition from that made from the unwilted crop.

The chloride content of some of the silages in which salt was used as a preservative was above the optimum for reasonable intake of salt, and in some cases it was excessive.

Losses in total nitrogen, as indicated by determining this element in both the fresh and the dried, ground samples, were very variable, being much higher in the silages than in the corresponding green crops.

Certain preservatives (molasses and ground wheat) tended to minimize this loss.

The chromatographic procedure for determining carotene gave results consistently somewhat lower than those obtained by the official phasic separation procedure.

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THE RELATION BETWEEN NUMBER OF EARS OPENED AND THE AMOUNT OF GRAIN TAKEN BY REDWINGS IN CORNFIELDS ¹

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INTRODUCTION

Damage to corn (*Zea mays* L.) by flocks of the eastern redwing (*Agelaius phoeniceus phoeniceus* (L.)) occurs in local areas over a large part of the United States. The pattern of damage in a cornfield at any particular moment is the expression of certain feeding habits of birds in flocks. This paper presents the results of an attempt to describe the progressive increase of damage in algebraic terms and to test the validity of the equations developed by application to data gathered in damaged fields. The work was undertaken to obtain a better understanding of the biology of redwing feeding in cornfields as part of an experimental testing of bird-scaring devices. The tests were made in collaboration with H. A. Cardinell, during four summers, 1939-42.³ It is assumed that these records of terminal damage in a number of fields are equivalent to records at successive stages of damage in a single field, at least as far as the relation between number of ears opened and amount of grain taken is concerned.

The diet of redwings is composed largely of weed seeds and insects. However, when the developing grain of the corn is in the soft or milk stage, the birds may attack the fields and continue their raids until the grain has become hard and ripe. The actual damage caused by the birds consists in ripping open the husks of the ears and removing some of the grain. The amount of grain taken from an ear by one bird at one visit is small, usually less than 5 percent of the grain in the ear. When feeding, the redwings flutter from cornstalk to cornstalk, stopping to feed at an ear for only a short time. Redwings feed in flocks of up to several hundred birds, but within a feeding flock relatively few birds seem to be actually eating at any one moment. Often an undisturbed feeding flock will pass slowly across a cornfield, the individual birds stopping to feed for a few moments while the flock as a whole progresses. A feeding flock often remains a moderately compact unit, and at alarm the birds may rise into the air together. Redwings appear to be easily disturbed, and often a flock will rise from one cornfield and fly to feed in another. After a short time the same flock, or another one, may be back in the first field, feeding again. Thus, a single bird does a small amount of feeding on each of a large number of ears, apparently spread over a considerable area of the countryside. Consequently, the total damage in any one field is the sum of a large number of small amounts of

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³ CARDINELL, H. A., and HAYNE, D. W. CORN INJURY BY RED WINGS IN MICHIGAN. Mich. Agr. Expt. Sta. Tech. Bul. [In press.]

grain eaten during a period of several weeks while the grain is ripening.

Redwings show a distinct preference for certain classes of ears of corn. In any field, the first ears to be damaged are those that first reach the milk stage. The ears of greater length and diameter, that is, the better ears, are apparently preferred. There may well be other criteria for preference.

DERIVATION OF EQUATIONS

The existence of preferred classes of ears within a field will be ignored for the moment, to be brought into the picture later. A uniform field is assumed first, where every ear has the same chance of being selected.

If n feeding birds are in a field containing N ears, and each bird damages one ear, the proportion of the ears damaged (x) will be

$$x = \frac{n}{N}$$

and the proportion not damaged (x') will be

$$x' = 1 - \frac{n}{N}$$

If selection is at random, the proportion not damaged after v visits will be

$$x' = \left(1 - \frac{n}{N}\right)^v$$

from which

$$v = \frac{\log x'}{\log \left(1 - \frac{n}{N}\right)} \quad (1)$$

If each bird takes b (for "bite") ears at each visit (as explained previously, b is probably small, much less than 1), then the flock will take nb ears at each visit. In v visits the flock will take a certain proportion of the grain in the field, represented by y in the equation

$$y = \frac{nbv}{N} \quad (2)$$

Substituting the value of v from equation (1) in equation (2)

$$y = \frac{nb \log x'}{N \log \left(1 - \frac{n}{N}\right)}$$

which may be stated in the form

$$y = \frac{b \log x'}{\log \left(1 - \frac{n}{N}\right)^{\frac{N}{n}}} \quad (3)$$

The denominator of (3) approaches a limit when N is many times as large as n , for the quantity

$$\left(1 - \frac{n}{N}\right)^{\frac{N}{n}} \quad (4)$$

when expanded takes the form

$$1 - \frac{\left(\frac{n}{N} \frac{N}{n}\right) + \frac{N}{n} \left(\frac{N}{n} - 1\right) \left(\frac{n}{N}\right)^2}{2} - \frac{\frac{N}{n} \left(\frac{N}{n} - 1\right) \left(\frac{N}{n} - 2\right) \left(\frac{n}{N}\right)^3}{2 \cdot 3} + \\ \frac{\frac{N}{n} \left(\frac{N}{n} - 1\right) \left(\frac{N}{n} - 2\right) \left(\frac{N}{n} - 3\right) \left(\frac{n}{N}\right)^4}{2 \cdot 3 \cdot 4} - \text{etc.}$$

The value of the sum of the terms of this expansion is approximately 0.3660 when $\frac{n}{N}$ is 0.01; 0.3670 when $\frac{n}{N}$ is 0.005; and 0.3679 when $\frac{n}{N}$ is so small as to have no effect on the value. As $\frac{n}{N}$ approaches zero, equation (4) approaches the value of e^{-1} .

In field observations flocks of birds larger than several hundred individuals were rarely observed feeding in cornfields, although larger flocks might often be seen at the roosting areas. The number of ears in a single acre of corn may be estimated as 10,000 or more. Therefore the quantity (4) may safely be used as a constant, of magnitude about 0.367. Substituting in equation (3)

$$y = \frac{b \log x'}{\log .367}$$

which reduces to

$$y = -2.30b \log x' \quad (5)$$

Since X' is the proportion of ears not damaged, equation (5) is more useful in the form

$$y = -2.30b \log (1-x)$$

or

$$y = k \log (1-x) \quad (6)$$

where x is the proportion of ears damaged and k is $-2.30b$.

Thus far we have assumed that selection of ears in a field is at random. However, as previously stated, this is not true over a whole field. Therefore let equation (6) refer only to one class within which there exists uniform probability of selection. One such class might be thought of as containing all ears about 6 inches long, of about the same diameter, about the same stage of maturity, and uniform in other respects. There will be a number, c , of these classes in a field, each class distributed over the whole field, extending side by side with all the other classes which are spread over the same area. Any one class will have a relatively small number of ears, such as N_1 , and the sum of all the ears in all the classes will, of course, be the total

number of ears in the field, or N . The various classes will not necessarily contain an equal number of ears.

The fact that the number of ears in any one class may approach the total number of birds in a flock brings up the question whether the fraction $\frac{n_1}{N_1}$, pertaining to class 1, is still small enough to make equation (4) a constant. Since any one class of ears may be widely scattered over the whole field, since the feeding flock is concentrated in only a part of the field, since only part of the birds in a flock seem to feed at any moment, and since the feeding birds are attacking at one time a number of classes of ears, it is probable that n_1 , the effective number of birds attacking class 1, will be quite small. Thus the conditions under which (4) is approximately constant will be maintained, and the course of damage within each class may be described by equation (6), as applied to class 1,

$$y_1 = k \log (1 - x_1)$$

The quantity k will be the same throughout all classes.

The actual amount of grain taken from class 1 will be

$$N_1 k \log (1 - x_1)$$

and the total amount of grain taken from the whole field will be the sum of as many such terms as there are classes. Dividing the total amount of grain taken by the total available ears, N , gives y , the proportion of the grain of a field taken by the birds; that is,

$$y = \frac{N_1 k \log (1 - x_1) + N_2 k \log (1 - x_2) + \dots + N_c k \log (1 - x_c)}{N}$$

or

$$y = k \left[\frac{N_1 \log (1 - x_1) + N_2 \log (1 - x_2) + \dots + N_c \log (1 - x_c)}{N} \right]$$

The right-hand side of this last equation is k times the logarithm of the weighted geometric mean of the proportion of the various classes of ears not damaged. Such a weighted geometric mean is not available. However, the figure at hand, the weighted arithmetic mean, may serve as an approximation of the geometric mean, although known to be somewhat larger.⁴ In the one case where data were available, in a sample of 1,088 ears, the weighted geometric mean of proportion of ears damaged in 60 size classes was 0.896, while the weighted arithmetic mean of the same classes was 0.907. Thus the form of the generalized expression remains the same as (6), although now assumed to refer approximately to a whole field instead of a single class within the field.

APPLICATION OF METHOD TO THE FIELD RECORDS

The field records consist of 198 estimates of damage made in fields during the seasons of 1941 and 1942. In each field an estimate was made by classifying about 200 ears into 5 groups according to damage and then computing an average of the damage per ear. The percent-

⁴ SIMPSON, G. G., and ROE, A. QUANTITATIVE ZOOLOGY. 414 pp., illus. New York and London. 1939. (See pp. 102, 146.)

age of ears opened was computed from the same field record. These 198 estimates have been reduced to 63 items by arranging them in groups according to the proportion of the ears opened and using the mean of each group as a single item. Each group contains the records of all fields falling within a class of 0.01 of the ears opened. For example, there are 4 fields having between 0.195 and 0.205 of the ears opened (20 percent of the ears), and these 4 fields average 0.034 of the grain removed (3.4 percent). The number of field records in the different classes varies from 26 estimates in the class of 0.01 of the ears opened to single records in a number of cases. In general, the less the damage the more numerous the field records.

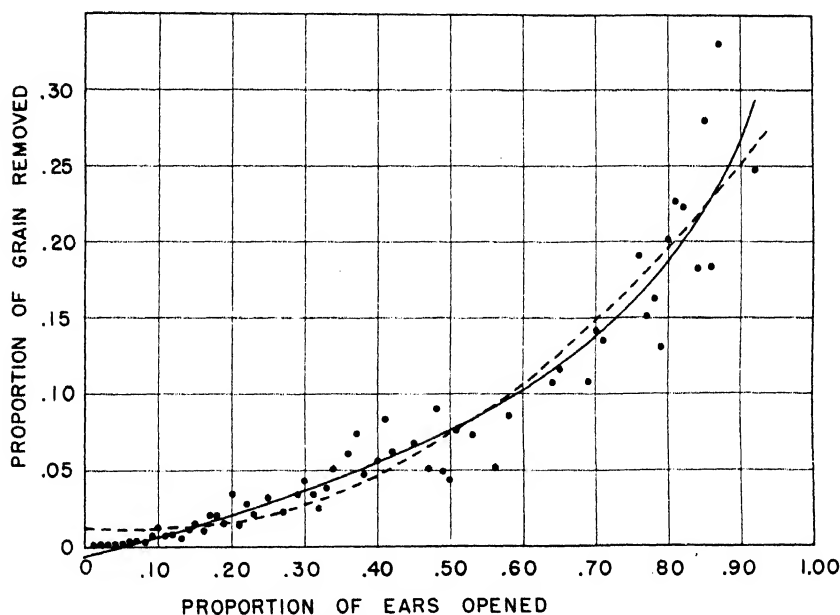


FIGURE 1.—Proportion of ears opened and proportion of total corn crop removed by redwings, based on 192 estimates of damage. Each dot represents the average of all fields having the same proportion of ears opened. The solid line is the curve derived from equation (7), $y = 0.0062 - 0.2745 \log (1-x)$, and the broken line is derived from the equation $y = -0.0132 - 0.0621x + 0.3633x^2$.

This combining of the data into classes is reflected in figure 1 in an apparent difference in variability at different levels of damage. This difference is, at least in part, an illusion caused by the averaging of more values for certain points than for others. This is not an important difficulty in this study, however, since the variability of the data is not of primary interest.

The 63 items (group means) were fitted to the derived curve (6), using logarithms and the method of least squares as ordinarily applied to fitting a straight line.⁵ The equation of the curve derived is

$$y = -0.0062 - 0.2745 \log (1-x) \quad (7)$$

⁵ RICHARDSON, C. H. AN INTRODUCTION TO STATISTICAL ANALYSIS. Edn., 312 pp., illus. New York. 1935. (See p. 177.)

The intercept of this curve on the y axis is not located at zero where it should be,⁶ but at -0.0062 (fig. 1). Consequently, use of the curve for translating values of proportion of ears opened to their theoretical equivalents in proportion of grain taken does not give satisfactory estimates for the range of from 0.01 to about 0.07 of the ears opened. The absolute deviations of the curve from the observed data are small, but the curve gives small negative values for the lower part of this range, a result which is biologically impossible.

The data in the range of 0.01 to 0.07 of the ears opened were fitted to the derived curve (6) independently, giving the somewhat different equation of

$$y = 0.0005 - 0.1081 \log (1 - x) \quad (8)$$

In this case the departure of the intercept on the y axis from zero is only 0.0005.

In the curve (7) fitted to the entire set of 63 items, a value of -0.2745 for k means a value of 0.119 ears for b (or about 12 percent of an ear per bird visit), since $k = -2.30b$. There are no field observations at hand to indicate whether this value is reasonable for the upper range of damage. It is known that 12 percent would be too high a value for the extreme lower end of the curve, for in field notes the first damage recorded on any ear resulting from the bird's visit when the ear is first opened was almost always less than 5 percent. However, the curve fitted to the whole set of data is in obvious disagreement with the data from the extreme lower part of the range, and the curve fitted to this lower range gives a value of 0.1081 for k with a corresponding value of 0.047 for b . This value of b is in much closer agreement with the field notes. There is thus a suggestion from these data that the birds may take a smaller amount of grain from ears that are just being opened than they do later, after damage has been started.

A parabola has been fitted to the 63 items by using the normal equations and the method of least squares.⁷ This curve is shown, along with the theoretically derived curve (7), in figure 1. The equation of the parabola is

$$y = 0.0132 - 0.0621x + 0.3633x^2.$$

For purposes of comparison a straight line has been fitted to the data. This line is not shown in figure 1. The equation of the straight line is

$$y = -0.0317 + 0.2621x.$$

Using the method outlined by Rider, a comparison was made of the closeness of fit to the data of the line, the parabola, and the derived curve (7).⁸ This method of comparison shows that either curve is better than the straight line, with a high degree of significance attached to the difference. Comparison of the parabola with the theoretically derived curve shows that although the theoretical curve fits the data more closely than does the parabola, the difference is not significant.

⁶ When no ears have been opened no damage can have been done; negative damage is impossible.

⁷ See footnote 5, p. —. (See p. 192.)

⁸ RIDER, P. R. AN INTRODUCTION TO MODERN STATISTICAL METHODS. 220 pp., illus. New York. 1939. See pp. 120 and 124.)

Reasoning on biological grounds, however, it is felt that the theoretically derived curve has points of advantage over the parabola. The intercept of the parabola is at 0.0132 on the y axis. The minimum value of y is not at zero on the X axis, where it should be biologically, but at about 0.09, where the predicted value of y is 0.0106. The greatest deviation, however, from the facts as observed in fields is found in the parabola predicting a value of 0.314 of the grain taken when all the ears have been opened. Although none of the field records shows that more than 0.92 of the ears were opened, the trend of the data at this point does not suggest that the birds opened all of the ears in any field with as little as 0.314 of the grain taken. It is obviously dangerous to argue beyond the limits of the data at hand, but the field observations appear to support the derived curve against the parabola.

Field notes for the seasons 1939 and 1940 were recorded only in terms of the proportion of ears opened. The derived curve, as stated in equation (7), has been used in translating these records to the approximate equivalent in proportion of grain removed.⁹ The results of this conversion of units seem satisfactory.

In this paper is presented a study of the relation between the proportion of ears of corn opened and the proportion of grain taken by redwings. The relationship was found to be fairly close, though not linear. Hence, the greater the number of ears opened the greater the loss of grain. There appears to be no necessity for assuming that birds return to an ear which has once been opened, merely because the ear has been opened.

Field observations and theoretical considerations support the belief that redwing selection of ears of corn within a cornfield is at random within certain preference classes. The field data conform to a curve derived upon these assumptions as closely as may be expected, in view of the limitations of the method used for measuring damage.

⁹ See footnote 3, p. 289.

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LATEX-TUBE AREAS OF THE ROOTS AND LEAVES OF THE RUSSIAN DANDELION¹

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INTRODUCTION

The distribution of the latex tubes and their appearance in cross section of the root of the Russian dandelion (*Taraxacum kok-saghyz* Rodin) have been shown by Artschwager and McGuire² in their morphological and anatomical studies of this plant. The object of the present investigation was to determine whether the percentage of latex-tube area in any one plant is sufficiently uniform in its various roots and leaves to justify the collection in the field of one leaf and one secondary root for the determination of latex-tube area. A technique was developed for this work by which the latex tubes could be clearly differentiated in the cross sections and thus could be easily counted and measured with the microscope.

TECHNIQUE USED IN THE INVESTIGATION

1. Segments of roots and petioles 3 mm. in length were fixed 24 hours in Flemings fluid (1 percent chromic acid 50 cc., 10 percent acetic acid 10 cc., 2 percent osmic acid 10 cc., distilled water 30 cc.). Air was pumped out of the segments when they were first placed in the fixing solution.

2. Washed 12 hours in running water.

3. Placed in 50 percent alcohol until ready for sectioning.

4. Sections cut on a hand microtome, the thickness of the sections ranging from 60 to 70 microns.

5. Stained 12 hours in saturated Calco Oil Blue NA in 50 percent ethyl alcohol.

6. Washed in water.

7. Mounted in Clearcol.

Since in the root the latex tubes are quite uniformly distributed in the region surrounding the xylem core, the tubes were counted in a quarter of that region and the number multiplied by 4 to get the total number of tubes in the entire cross section. The diameter of 30 tubes selected at random was obtained by the use of an eyepiece micrometer scale and these diameters were used in getting the average tube area. By multiplying the average tube area by the number of tubes, the total latex tube area was obtained. The total area of the cross section was calculated. By using the total cross-sectional area and the total latex-tube area the percentage of latex-tube area was obtained. The size of the tubes in the different regions outside

¹ Received for publication October 24, 1944. This report is based upon investigations carried on by the author between June 20 and September 20, 1943, as Bankhead-Jones Project 1K of the Botanical Section of the Experiment Station of Michigan State College.

² ARTSCHWAGER, E., and MCGUIRE, R. C. CONTRIBUTION TO THE MORPHOLOGY AND ANATOMY OF THE RUSSIAN DANDELION (*TARAXACUM KOK-SAGHYZ*). U. S. Dept. Agr. Tech. Bul. 843, 24 pp., illus. 1943.

the xylem core varied, the smaller tubes being next to the xylem core and next to the periderm, and the larger tubes in the intermediate region (fig. 1, *A*).

For studying the latex tubes in the petiole (fig. 1, *B*), basal segments 3 mm. in length were fixed. Ten leaves were selected at random from

each plant and about 50 sections were cut from the segment of the petiole of each leaf and mounted at random on a slide. The number of latex tubes was determined in the midvein of 20 sections selected from the 50 sections. In making the selection for counting the latex tubes a mechanical stage was used. The sections were selected in sequence as they moved across the field.

PRESENTATION OF RESULTS

The results of the investigations are shown in tables 1 to 3. A comparison of the number of latex tubes in 10 leaves taken from 1 plant is shown in table 1. The percentage of latex-tube area at various levels in the primary root is shown in table 2. A comparison of the total latex-tube area in the primary root with that in its large secondary roots is shown in Table 3, and this table also shows the total latex-tube area in the 7 secondary roots arising from a primary root.

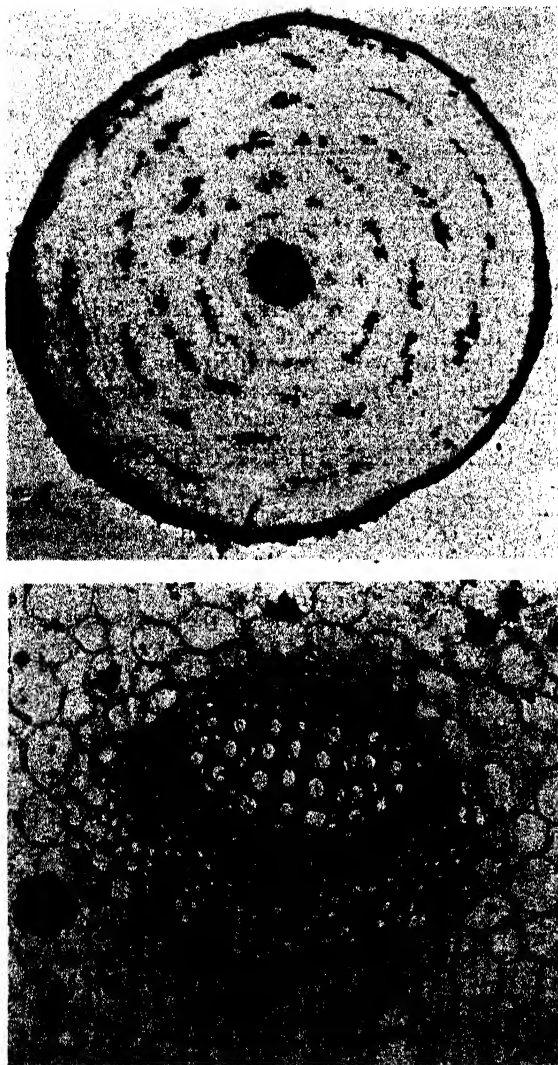


FIGURE 1.—*A*, cross section of secondary root, showing distribution of latex tubes; *B*, cross section of midvein of petiole, showing distribution of latex tube in the form of an arc along the edge of the phloem.

The 10 leaves from a single plant showed a marked variation in the number of latex tubes in each midvein of the 10 petioles and also at various levels in the same petiole; for example, as shown in table 1, the number of latex tubes in the midvein of the petiole of 10 leaves

ranged from 2 to 29. In similar group of 10 leaves from another plant the range was 8 to 25. In a primary root there is evident a variation in the total latex-tube area at various levels, as shown in table 2, the greatest being 31 mm. from the crown. A comparison of the total latex-tube area in the primary root with that in its secondary roots showed a marked variation; for example in table 3 the primary root had 0.16 percent latex-tube area while its 5 secondary roots had from 0.27 to 2.06 percent. Similar observations of primary and secondary roots in other Russian dandelion plants showed the following: Primary 0.84 percent, its 3 secondaries 0.71 to 1.09 percent; primary 1.35 percent, its 3 secondaries 0.43 to 1.35 percent; primary 0.96 percent, its 5 secondaries 0.6 to 1.03 percent; primary 0.7 percent, its 2 secondaries 0.76 and 0.86 percent; and primary 0.68 percent, its 5 secondaries 0.16 to 1.89 percent. In table 3 the 7 secondary roots arising from 1 primary root showed a variation in latex-tube area ranging from 0.14 percent to 1.56 percent.

TABLE 1.—Number of latex tubes in cross section of midvein of petioles of 10 leaves taken from 1 plant

Leaf No.	Latex tubes in section No.—																				Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	19	15	15	15	13	14	16	13	18	19	16	15	16	12	14	19	14	2	9	6	14
2	18	11	14	17	12	16	15	13	16	20	13	16	16	18	18	17	22	17	14	11	16
3	8	15	11	16	14	14	11	14	10	15	12	14	15	15	12	10	10	15	12	13	13
4	13	15	15	18	19	13	20	21	11	18	16	14	19	14	14	10	18	13	13	21	16
5	21	11	25	13	29	16	17	25	15	22	20	10	8	11	14	8	18	22	18	16	17
6	14	14	6	10	6	19	15	21	19	12	20	13	19	22	14	17	16	10	9	8	14
7	12	16	16	14	18	13	14	17	16	14	20	20	22	13	23	21	18	23	24	14	17
8	21	13	15	18	20	14	18	24	22	15	18	23	21	13	14	15	16	23	22	24	18
9	16	19	19	18	21	19	18	12	18	16	17	19	18	18	13	17	18	16	19	16	17
10	8	8	22	17	13	12	17	22	9	21	16	9	13	19	18	17	14	12	11	10	14
Average																					15.6

TABLE 2.—Total latex-tube area at different levels in the same primary root

Distance from crown (mm.)	Area of root section	Number of latex tubes	Average area of latex tube	Total area of latex tubes	Percent of latex-tube area in cross section
	a^2		a^2	a^2	
3	8, 272, 571	628	25	15, 769	0.19
6	9, 142, 798	532	33	16, 222	.18
9	8, 702, 245	544	38	20, 705	.23
12	7, 648, 457	456	31	13, 999	.18
15	6, 478, 166	564	36	20, 524	.33
18	6, 089, 522	588	44	25, 731	.42
21	6, 662, 656	556	24	13, 511	.20
25	6, 662, 656	420	28	12, 007	.18
28	6, 089, 522	476	43	20, 706	.34
31	7, 445, 858	572	69	39, 577	.53

TABLE 3.—*Total latex-tube area in the primary root and its secondary roots and in the secondary roots arising from 1 primary root*

PRIMARY ROOT AND ITS SECONDARY ROOTS

Part of root system	Area of root section	Number of latex tubes	Average area of latex tube	Total area of latex tubes	Percent of latex-tube area in cross section
	μ^2		μ^2	μ^2	
Primary root	18,430,888	1,828	107	195,925	0.16
Secondary A	1,461,032	196	91	17,914	1.23
Secondary B	13,810,857	258	166	37,734	.27
Secondary C	1,190,834	180	184	33,197	2.03
Secondary D	7,957,455	412	167	68,878	.87
Secondary E	2,531,917	376	138	52,046	2.06

SECONDARY ROOTS ARISING FROM 1 PRIMARY ROOT

Secondary A	5,190,017	504	141	81,099	1.56
Secondary B	7,270,815	484	138	66,826	.92
Secondary C	4,302,257	296	93	27,590	.64
Secondary D	2,228,719	252	125	31,467	1.41
Secondary E	3,634,303	456	125	57,091	.15
Secondary F	4,830,873	500	101	50,430	.14
Secondary G	4,302,257	364	102	36,957	.85

SUMMARY

A study of the Russian dandelion has been made to determine (1) the number of latex tubes in petioles of different leaves from the same plant, (2) the total latex-tube area at different levels in the same primary root, (3) the total latex-tube area in the primary root as compared with that in its secondary roots, and (4) that in the secondary roots arising from a single primary root.

The results of these studies fail to show any trend that would enable one to determine the total latex-tube area in a root system by examination of the primary or one secondary root of the system or one petiole of the plant.

SOME ASPECTS OF THE AERIAL DISSEMINATION OF SPORES, WITH SPECIAL REFERENCE TO CONIDIA OF *SCLEROTINIA LAXA*¹

By E. E. WILSON, *associate plant pathologist*, and G. A. BAKER, *assistant statistician*
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INTRODUCTION

Spread of the brown rot blossom disease by means of air-borne conidia of the casual fungus *Sclerotinia laxa* Ader. and Ruh. from well-defined centers of infection has been observed in at least 15 apricot orchards in California during the past 5 years. In 7 of these the number and disposition of the trees furnishing and receiving the conidia were such that the incidence of blossom infection could be compared quantitatively at different distances from the nearest source trees. Attempts were then made to determine the major factors influencing wind dissemination of spores, and to correlate the results with the data on disease spread.

ADAPTATION OF *SCLEROTINIA LAXA* TO WIND DISSEMINATION

Conidia of *Sclerotinia laxa* are produced on sporodochia which develop in large numbers during late winter on blossoms and twigs infected the previous spring. According to measurements made during these studies, the spores average 14μ long and 12μ broad. When mature they were detached from the sporodochia in fairly large numbers by an air current moving at 2.4 m. p. h. In one test conducted according to the method outlined by McCubbin (11)³, their rate of settling in still air averaged about 0.36 cm. (0.14 inch) per second. Accordingly, conidia liberated at a height of 6 feet and falling freely (not affected by cross currents in the air stream) would be carried about 3,770 feet from the source by a 5 m. p. h. wind before reaching the ground. Because wind movement is seldom steady enough to permit a free fall, such a comparison is useful only to illustrate the ease with which even a gentle breeze may carry the spores. Updraughts moving at a rate slightly greater than the spores' terminal velocity will bear them upward and so permit their dissemination over great distances. There is ample evidence regarding the great distances to which spores much larger than those of *S. laxa* are carried by air currents (12).

¹ Received for publication October 2, 1944.

² Grateful acknowledgment is due Dr. F. A. Brooks, California Agricultural Experiment Station, for advice concerning atmospheric properties; to Dr. Katherine Esau, California Experiment Station, for translating Stepanov's article; and to Dr. K. Starr Chester, Oklahoma Agricultural and Mechanical College, for translating Boevsky's paper.

³ Italic numbers in parentheses refer to Literature Cited, p. 326.

SPREAD OF THE BROWN ROT BLOSSOM DISEASE IN APRICOT ORCHARDS

During the testing of monocalcium arsenite (20) as an eradicant spray against the conidia of *Sclerotinia laxa*, fairly large blocks of apricot (*Prunus armeniaca* L.) trees were rendered comparatively free⁴ of inoculum. Since the spray was applied in January, it gave little or no protection to blossoms; and whenever air-borne conidia were brought into the sprayed trees from nearby sources, blossom infection developed abundantly (20). In such cases gradients of blossom infection developed in the recipient trees (sprayed) adjacent to the source trees (unsprayed).

GRADIENTS OF BLOSSOM INFECTION IN THE VICINITY OF SPORE SOURCES

In 1939 three and in 1940 four such gradients were studied in the Sacramento Valley. Data on the amount of blossom infection were secured in each recipient and each source tree in these areas. Figure 1 illustrates the situation in one orchard. The recipient trees adjoined the source trees on the north, east, and south. In trees east, south-east, and south of the source, never more than 1 percent of the blossoms

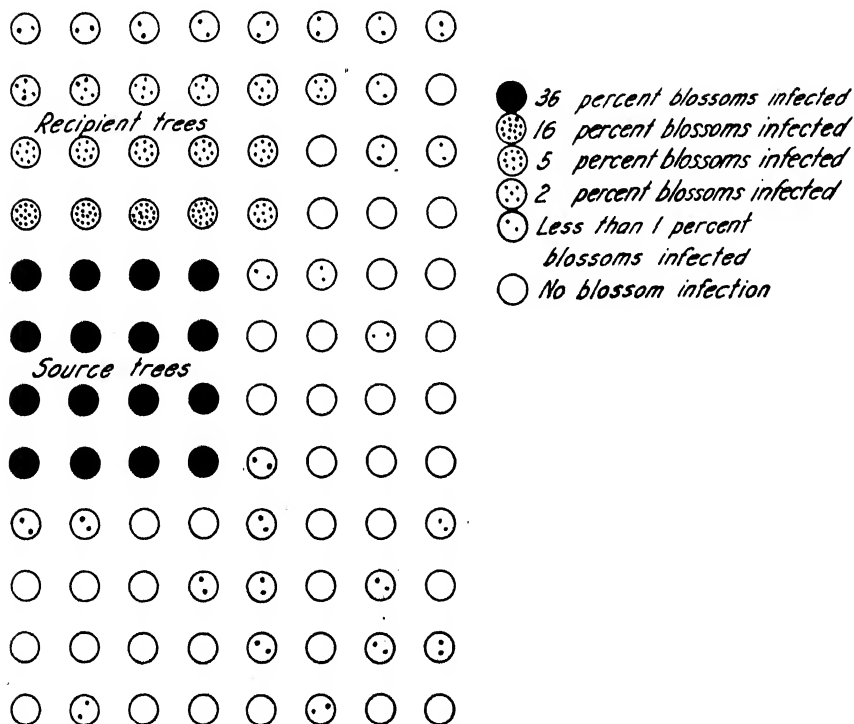


FIGURE 1.—Gradations in the amount of blossom infection by *Sclerotinia laxa* in inoculum-free (recipient) apricot trees located at different distances north of trees (source) in which conidia of the fungus were abundant.

⁴ Free of inoculum in the sense that the number of effective conidia remaining in the trees sprayed with monocalcium arsenite incited only insignificant amounts of blossom infection.

were involved, but in those whose centers were 22, 44, 66, and 88 feet north of the centers of the northernmost trees in the source block, the blossom infection averaged, respectively, 16, 5, 2, and 1 percent. Interestingly enough, all seven gradients observed in the 2 years extended north from the source trees. The spores responsible for this blossom infection must, therefore, have been disseminated by south winds.

The level of disease development varied somewhat among the seven lots of source trees. Such variability resulted from differences in one or more of the following factors: (1) The number of conidia available for dissemination from the source, (2) the number of spores detached from the sporodochia by winds, (3) viability of the conidia, and (4) weather conditions that influence blossom infection. As they stood, therefore, the data were not in a form for comparing disease development between orchards or between years. When, however, the level of disease in each lot of source trees was given equal value, such as 100, and the level of disease in each lot of recipient trees was calculated as a percentage of that at the appropriate source, variabilities resulting from the factors listed above were minimized or eliminated.

The most noticeable result of such a treatment (table 1) was the separation of the data into two groups with respect to the rate that blossom infection diminished with increased distance from the source. The most rapid diminutions occurred in the three gradients observed in 1939, where at 22, 44, 66, and 88 feet from the source tree the blossom infection averaged 39, 21, 12, and 6.5 percent, respectively, of that at the source, as against corresponding values of 55.5, 40.0, 28.0, and 23.0 percent for the four 1940 gradients.

TABLE 1.—Comparative incidence of blossom infection by *Sclerotinia laxa* in trees producing sparse inoculum but located at different distances from trees producing an abundance

Year and orchard	Incidence of disease in recipient trees at stated distances (feet) from the source, expressed as a percentage of the incidence at the source			
	22 feet	44 feet	66 feet	88 feet
1939:				
A	44.4	16.0	9.0	5.1
B	40.1	25.6	11.7	6.3
C	35.1	20.0	13.0	7.7
1940:				
D	56.8	45.1	33.4	26.7
E	55.0	39.0	26.9	19.3
F	45.0	33.3	22.2	19.9
G	61.0	26.8	19.5	15.6
Average, ¹ 1939, A to C	39.0	21.0	12.0	6.5
Average, ¹ 1940, D to G	55.5	40.0	28.0	23.0

¹ These averages, computed from the original data, are not in all cases equal to the means of the percentages shown above.

Similar infection gradients have been reported for several other fungus diseases, but in comparatively few of these are quantitative data given. Examples of such gradients are furnished by Newhall (13) for the spread of onion downy mildew and by Boevsky (1) for the spread of wheat leaf rust.

RELATION OF GRADATIONS IN INCIDENCE OF THE DISEASE TO GRADATIONS IN THE DENSITY OF AIR-BORNE SPORES

Unilateral variations in disease development, as illustrated by the cases cited above, suggest that corresponding unilateral variations in the density⁵ of air-borne spores existed when the spores were disseminated. Dissemination undoubtedly occurred throughout the late winter and spring, and in various directions from the source trees; but effective dissemination (dissemination resulting in establishment of the disease at different distances northward from the source trees) must have occurred in a period when winds were from the south. According to table 2 the blossoming period in 1939 extended from February 25 to March 17; that in 1940 from February 25 to March 20. Rain fell between March 6 and 9, 1939, when the apricot trees were in full bloom; and between February 25 and February 29, 1940, extending into the early part of bloom. Except for traces of rain the weather was dry throughout the remainder of both periods. Apparently, therefore, most or all of the blossom infection was initiated between March 6 and 9 in 1939 and between February 25 and 29 in 1940. Effective dissemination probably occurred before or during these rains.

Since the recipient trees developing blossom infection were located north of the source trees, the effective conidia must have been disseminated by south winds. Records of wind direction and velocity in the orchards were not available; but, according to weather stations in the districts where the orchards were located, south winds prevailed during the rainy periods of both years (table 2). In both years, furthermore, north winds prevailed for a time immediately before the rains started. Thus, effective dissemination does not seem probable in any part of the blossoming period before infection except during the rainy days (March 6 to 9, 1939; February 25 to 29, 1940).

The Weather Bureau ordinarily records wind movement at some distance from the ground; and, in consequence, the velocity which it reports will exceed that at orchard level. For the writers' purpose, however, the difference in velocity between the two periods of spore dissemination is just as important as the exact velocity in the orchard. According to the Sacramento station the average velocity between March 6 and 9, 1939, was 8 miles per hour (table 2), and the average velocity between February 25 and 29, 1940, was 15 miles per hour. During 1940, when the decrease of blossom infection in disease gradients was slow, velocities were 1.88 times greater than in 1939, when the rate of decrease was more rapid. By inference, therefore, the differences in wind velocity account for the observed differences in spore dissemination.

In all probability, the aerial density of effective spores was correlated with the unilateral development of the disease. Boevsky (1) demonstrated such a correlation for leaf rust of wheat caused by *Puccinia triticina*. Apparently, however, several factors may alter the quantitative relations between density of spores and level of disease development. By assumption, the amount of blossom infection is proportional to the number of conidia reaching the blossoms; but this proportionality is not necessarily constant. Four factors were

⁵ For reasons apparent later, density is defined as the number of air-borne spores that pass through a unit area of a plane perpendicular to the direction of the wind (mean wind direction).

mentioned earlier, all of which could affect the relative extent of disease spread between orchards and between years. The effect of these factors on the data in table 1 was minimized by mathematical treatment. There remain other factors that are not affected by this treatment. The first is the possibility that the disease in recipient trees may have been increased by conidia produced on currently infected blossoms. According to Lambert (9), in stem rust of wheat the disease zones near barberry bushes, though originating in infection by aeciospores from the bushes, are extended to a much greater degree by urediospores produced on the wheat itself. In brown rot, however, the susceptible period of the host is so short that secondary blossom infection apparently does not occur to any appreciable extent (20).

The second factor, illustrated by Boevsky's (1) results on leaf rust of wheat, is the tendency for the lesions or comparable foci of infection on recipient hosts nearest the source of spores to become so numerous as to introduce a considerable error in counts of their numbers. This error probably reaches appreciable proportions even before the mechanical difficulties of counting become acute. Since in the present studies the maximum infection in recipient trees nearest the source was only 27 percent of the total blossoms in the trees, there is no reason to suspect that these counts reflect the aerial density of spores less accurately than counts obtained at greater distances from the source.

A third factor is the rain that fell throughout both dissemination periods. Two effects of rain seem possible: (1) Reduction in the number of spores available for dissemination; (2) reduction in the distance the spores are carried by the wind. The first would be caused by rain washing the conidia from the source trees, and the second by rain washing the spores from the air. Effects of variations in the number of spores available for dissemination were minimized by mathematical treatment; but not the effects of rain on the distances to which spores were carried by the wind. While no definite answer concerning the latter effects can be given, rain probably did not cause the observed differences between the 1939 and 1940 disease data, inasmuch as rainfall during the effective dissemination period was heavier in 1940 than in 1939.

On the whole, therefore, the characteristics of the blossom-infection gradients reported in table 1 are believed to have been determined by differences in the aerial density of conidia disseminated from the primary source, the group of nonsprayed trees adjacent to the recipient trees. The remainder of this paper will discuss certain characteristics of aerial dissemination.

EFFECT OF VERTICAL AND LATERAL DISPERSION OF SPORES IN AIR CURRENTS ON THEIR DENSITY AT DIFFERENT DISTANCES FROM THE SOURCE

If, as indicated in the foregoing section, the aerial density of spores is affected by distance from the spore source and possibly by differences in wind velocity, the next feature to examine is the pattern of aerial dissemination. The first question concerns the vertical and lateral dispersion of air-borne spores as they are carried downwind. One need only observe dust or smoke to see that as these materials are blown

from the source the particles are scattered more and more in all directions transverse to the mean direction of the wind. Presumably the cause of this dispersion is the eddies or "turbulence bodies" in the air stream, which are known to be the means for transferring such air properties as temperature, carbon dioxide, and moisture from one point to another in the atmosphere (2, 4).⁶ Although turbulence cannot be discussed here in detail, some of its salient features will be mentioned.

According to records made with appropriate meteorological instruments, wind movement is seldom steady for any great length of time, but is marked by recurrent gusts and lulls, associated with cross-current eddies. The eddies vary in duration, but usually last for a few seconds only. Those of shorter duration appear to be distributed at random as regards their movement in directions transverse to the mean direction of the wind. According to Geiger (4), the movement of an individual eddy, if it could be followed, would seem fortuitous in the extreme; yet, in the aggregate, the movement of turbulence bodies through a given mass of air accomplishes a transfer of properties in much the same way as molecular diffusion in gases, though at a much greater rate.

The point that bears upon the present problem is the fact that airborne particles, such as the fungus spores acted upon by these eddies, probably tend to "diffuse" or become more and more widely dispersed as they are carried by the wind. Thus, at any given distance from the point at which the spores are liberated into the air, their density will depend upon the number liberated and upon the rate of dispersion. Considering for a moment a source of limited dimensions, such as a sporodochium or fruiting structure of *Sclerotinia lara*, one sees that if the spores are liberated at successive intervals individually or in clouds, there will be a progressive decrease in the number passing through equal areas of perpendicular planes located at increasing distances from the sporodochium. Whether or not this number bears any constant relation to the distance from the source depends upon whether or not the dispersion of the spores follows a consistent pattern. Probably the simplest relation between distance and spore density would occur if the progressive positions of successive clouds of spores formed sections of a cone whose apex was at the source. Judging from observations on a wind-blown column of smoke, the space dispersion of the particles is roughly conical. If spores behave in this way, their aerial density in plane section would decrease with distance as the area of the right-plane section of the cone increases. Since the area of the right-plane section of all cones increases in proportion to the square of the distance from the apex, spore density, in the sense defined above, would decrease in inverse proportion to the square of the distance. A true conical distribution would occur only if the spores dispersed in such a way that their density was the same at all points in the right-plane sections of the cone and if the right-plane sections were circular. As is shown later, neither of these conditions actually obtains; but no serious error results from assuming an ideal conical space distribution.

The cone, therefore, furnishes a geometric model for studying experimentally the dissemination of spores. Before the experimental results can be described, it is necessary to define certain terms used

⁶ The papers pertaining to turbulence in the atmosphere are too numerous to be listed here. Although many were examined by the writers, for the most part the excellent summaries by Brunt (2) and Geiger (4) were consulted.

in the following discussions. In the representation of a cone in figure 2, let O , the apex, be the source of spores; and let the right-plane sections, I, II, III, be located 5, 10, and 15 feet from the apex. Line P which passes through the "center," C , of each right-plane section is the "projection of the point of release" in the mean wind direction. As the spores are blown downwind they are also dispersed in all directions transverse to P , the "vertical dispersion" of course being above and below the "horizontal dispersion" to the right and left of P . Whenever, in the following discussion, a right-plane section of a cone or any plane perpendicular to the direction of the mean wind is considered, C is called the "center of the plane."

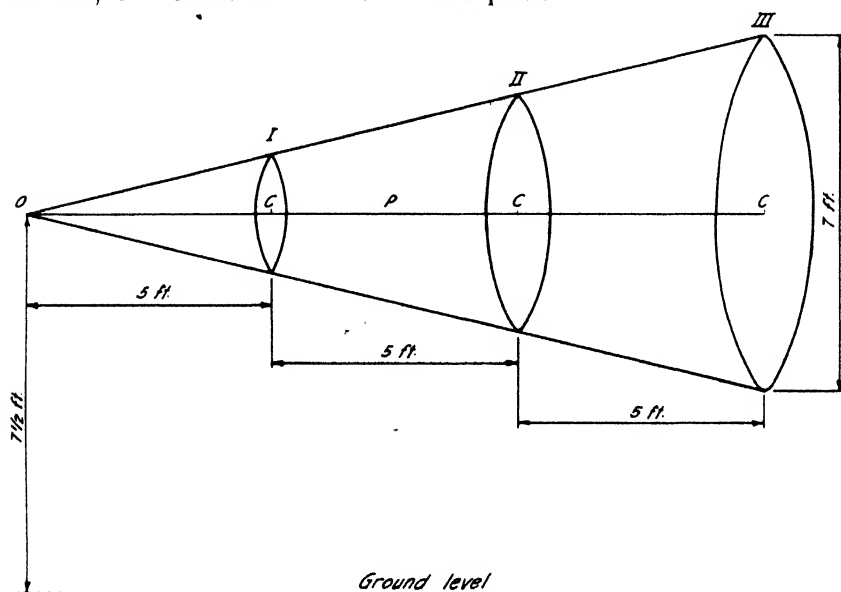


FIGURE 2—Geometric model for studying the relation of the intersecting plane density of air-borne spores to distance from the source.

To determine dispersion of the spores away from the center of the plane, one must determine their density at different points along vertical and horizontal axes of the right-plane sections, I, II, and III. Their array along these axes is termed "distribution," and this term is to be considered in its statistical sense.

DECREASE IN DENSITY OF AIR-BORNE SPORES WITH INCREASED DISTANCE FROM THE SOURCE

To determine the decrease in density of spores at increased distances from the source, a framework was constructed consisting of three slender upright supports (5 feet apart) with cross arms at a distance of 7.5 feet from the ground. On each cross arm were placed five oil-coated slides. Of these, four outlined the circumference of a horizontal cone with its apex 5 feet from the first support and its base on the last support. The fifth slide was placed in the center of the right-plane section of this cone (fig. 2, C). An experimental cone of these dimensions was selected for convenience, not because all the spores were expected to be disseminated within these limits.

Preliminary trials at releasing conidia of *Sclerotinia laxa* proved unsatisfactory because the spores adhered in clumps. It was also difficult to maintain the large supply of spores necessary for frequent tests. Spores of the clubmoss, *Lycopodium* sp., were then tried and found satisfactory. Although these are about 2.5 times greater in diameter than conidia of *S. laxa*, they are readily carried by ordinary air currents. Stepanov (18) reported their rate of fall in still air to be 2.14 cm. sec. They possessed the additional advantages of separating from each other when released into the air and of being easily identified. They can also be obtained in large quantities from commercial sources.

Tests were conducted as follows: The apparatus was oriented with the base of the cone in a downwind direction. A vane anemometer located 7.5 feet from the ground recorded the wind velocity throughout. The spores were released at the apex of the cone (7.5 feet from the ground) by gently expelling them from a small puff-type hand duster, the outlet of which was pointed at right angles to the mean direction of the wind. After successive puffs of spores had been released in this manner for 15 minutes, the slides were removed and examined. To avoid errors—since more spores are ordinarily caught near the edges of the slides than in the center—the same pattern was followed in counting the numbers in 50 microscopic fields on each slide. Inasmuch as the vaseline-cottonseed oil film appeared very uniform, errors arising from differences of adhesiveness between slides are believed to be insignificant.

Table 3 contains the results of 16 tests where the wind varied between 2.2 and 16.0 miles per hour. Since the number of spores released could not be determined with any reliable degree of accuracy, the number caught per given area at the first right section (5 feet from the source) was taken as the reference value which was used to calculate the expected density of spores at 10 to 15 feet from the apex, assuming their density to be the same at all points within sectional limits of the cone. The coefficients for determining the expected density at 10 and 15 feet are 0.25 and 0.111, respectively; that is, at 10 and 15 feet the number of spores per given area should be, respectively, 25 and 11.1 percent of that at 5 feet.

These proportional relations hold for any cone. Although many of the spores were carried outside the limits of the experimental cone, their density varied approximately in inverse proportion to the square of the distance from the source. In some tests the observed value was greater and in some less than the expected value. On the whole, however, the variations appear to be within the limits of experimental error. For various reasons it appears that a closer agreement between the two values might have been obtained had the distances from the source been greater. A material increase in distances between supports, however, would necessitate a framework too long to be easily shifted to meet changing wind directions.

The inverse-square relation between aerial density of spores and distance from the source also holds when but one part of the right section of the cone is considered—that at the center of the plane. Table 4 expresses the relative density of spores on center targets at 10 and 15 feet as a proportion of that on the center target at 5 feet from the source. The observed values vary around their respective

TABLE 3.—Comparison of the observed and expected density of air-borne spores of *Lycopodium* at 3 right-plane sections of the experimental cone, observed values are averages of results from 5 targets at each distance

Wind velocity (miles per hour)	Number of spores on 67 mm. ² at stated distances (feet) from source (apex of cone)			
	Data ¹	5 feet	10 feet	15 feet
2.2	{ Observed	629	230	111
	{ Expected	629	157	70
2.5	{ Observed	156	56	19
	{ Expected	156	39	17
3.5	{ Observed	170	30	
	{ Expected	170	43	
3.8	{ Observed	484	93	42
	{ Expected	484	121	54
3.8	{ Observed	347	158	48
	{ Expected	347	87	39
4.3	{ Observed	383	79	39
	{ Expected	383	96	43
4.4	{ Observed	591	143	
	{ Expected	591	148	
5.1	{ Observed	809	238	106
	{ Expected	809	202	90
5.3	{ Observed	550	168	58
	{ Expected	550	138	61
5.6	{ Observed	446	145	66
	{ Expected	446	112	50
8.5	{ Observed	1,396	361	165
	{ Expected	1,396	349	155
11.3	{ Observed	1,340	307	115
	{ Expected	1,340	335	149
12.1	{ Observed	478	130	
	{ Expected	478	120	
12.5	{ Observed	608	145	
	{ Expected	608	152	
12.5	{ Observed	618	145	
	{ Expected	618	154	
16.0	{ Observed	2,764	779	326
	{ Expected	2,764	691	307
Average	{ Observed	736	200	100
	{ Expected	736	184	94

¹ Expectancy at 10 feet is 25 percent and at 15 feet 11.1 percent of that at 5 feet

TABLE 4.—Density of air-borne spores of *Lycopodium* in a direct line 10 and 15 feet to windward of the point of release, values expressed as percentages of the density at 5 feet from the point of release

Wind velocity (miles per hour)	Number of tests averaged	Number of spores caught at stated distances (feet) for each 100 spores caught at 5 feet	
		10 feet	15 feet
2.0-2.5	2	34	10
3.1-3.8	3	32	11
4.1-4.4	3	25	11
5.1-5.9	4	26	12
6.6	1	22	7
8.1-8.5	2	31	14
11.3	1	24	10
12.1-12.5	3	25	10
13.4	1	29	13
16.0	1	32	12
Mean of 21 tests		28	11
Expected if dispersion is conical		25	11

means, which in turn are close to the theoretical values. Again the agreement between observed and theoretical is closest at the greater distance from the source of spores.

Heald, Gardner, and Studhalter (6), working with *Endothia parasitica*, and Lambert (9), working with *Puccinia graminis*, found the number of air-borne spores to decrease rapidly as distance from the source increased. Stepanov (18) studied the dissemination of *Tilletia tritici* and *Bovista plumbea* by releasing the spores at a given point 0.5 to 3 meters above the ground and catching them on glass slides placed face up on the ground at various distances downwind from the point of release. In three tests when wind velocity varied between 5 and 6 m. p. h. the numbers of spores caught at 10, 15, 20, and 40 m. were 24.1, 11.9, 7.99, and 1.2 percent respectively of the number caught at 5 m. When dissemination follows a conical pattern, the expected percentages should be 25.0, 11.1, 6.2, and 1.6 respectively. Boevsky (1) studied the dissemination of urediospores of *Puccinia triticina* from a field of winter wheat. His data are complicated by two facts: (1) The urediospores of *P. dispersa* from a nearby rye field were included in the counts at certain times; (2) the spring wheat surrounding the spore traps became infected by *P. triticina* and produced urediospores. If one omits from Boevsky's data the counts in which these complications occurred, the numbers of spores caught at 50, 100, and 200 meters from the edge of the source field were 322, 119, and 27 respectively, whereas the numbers expected when dispersion is conical should be 322, 80, and 20.

DISTRIBUTION OF AIR-BORNE SPORES IN THE VERTICAL DIRECTION TRANSVERSE TO THE MEAN WIND DIRECTION

Despite the concordance between experimentally observed results on spore density and the results expected if dispersion were conical, the distribution of spores within the cross-sectional area of the cone was not uniform. Their density was greatest at the center of the plane—that is, at the level of release—and decreased outward to the periphery of the experimental cone and beyond. Since the number of targets at each right-plane section was insufficient for showing the over-all vertical distributions of the spores the experimental apparatus was redesigned. Beginning 18 inches from the ground, 13 slides were placed at 1-foot intervals on each of the three vertical supports, which (as will be remembered) were 5 feet apart. Tests were conducted as follows: After the apparatus had been oriented to the direction of the mean wind, the spores were released at a point 5 feet from the first vertical support and at a height of 7.5 feet; that is, on the level with the center slide in each vertical series.

Table 5 gives results of the tests conducted at seven different wind velocities. The array of spores above and below the center of the plane was roughly similar. In gross aspects the distributions followed that of normal probability. In a given test the vertical distributions occurring at 5, 10, and 15 feet from the source differed noticeably; the dispersion of spores increased with distance. A measure of dispersion is afforded by the standard deviation of the distributions. The distributions obtained at all three distances in winds blowing 5.9 to 16.0 miles per hour and those obtained at 5 feet in winds blowing 3.8

TABLE 5.—Vertical distributions of air-borne spores of *Lycopodium* at successive distances from the point at which the spores were released; standard deviations express the degree of dispersion at the stated distances

Distance, in feet, above (+) and below (-) level at which spores were re- leased	Number of spores on 67-mm. ² area at stated wind velocity and at stated distances (in feet) from the source of spores																							
	3.8 m. p. h.			5.9 m. p. h.			6.4 m. p. h.			8.1 m. p. h.			8.5 m. p. h.			13.4 m. p. h.			16.0 m. p. h.					
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15			
+6	3	3	15	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	1	2		
+5	9	9	16	0	0	9	0	0	0	1	0	0	5	0	0	0	0	0	0	1	10			
+4	2	13	5	0	17	0	3	0	0	6	0	1	9	0	0	14	1	3	32	63	0			
+3	7	35	12	0	31	0	3	0	0	30	0	1	34	0	1	33	39	1	93	143	94			
+2	16	34	14	8	34	5	17	10	14	38	14	55	50	41	27	79	25	143	284	130	0			
+1	97	36	18	183	144	60	82	26	133	118	73	214	138	105	716	210	94	1,446	457	167	0			
0	172	27	27	932	206	104	317	64	25	632	230	103	941	228	105	241	158	81	699	277	152			
-1	90	77	12	87	108	65	34	41	27	328	157	81	241	123	79	241	158	81	699	277	152			
-2	48	40	15	6	24	23	1	13	33	79	39	18	51	44	11	61	51	44	137	97	0			
-3	17	15	13	1	3	16	0	1	19	31	0	15	11	1	5	19	2	49	48	0	0			
-4	17	5	12	0	0	0	0	0	2	0	0	1	3	0	0	0	0	6	4	0	0			
-5	4	3	9	0	0	0	0	0	1	0	0	1	4	0	0	0	0	0	0	0	0			
-6	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Mean (in feet) measured from the level of release (below - minus)	-0.28	0.10	2.90	0.08	0.19	0.36	0.13	0.24	0.21	-0.21	-0.25	-0.02	-0.02	0.03	0.26	-0.01	-0.16	0.28	-0.04	-0.02	0.16			
Standard deviation (σ) in feet	1.64	2.10	5.95	.52	1.13	1.95	.53	1.08	1.67	.73	1.23	1.80	1.84	1.29	1.84	.73	1.20	1.96	.76	1.37	1.85			

¹ This distribution is obviously incomplete and hence the estimation of the mean and standard deviation present technical difficulties. The estimates given here are obtained by applying the method of difference equation graduation for a stump of a normal distribution curve as explained by Carver (3).

miles per hour were considered to be complete, and the means and standard deviations were obtained in the usual manner. For the incomplete distributions at 10 and 15 feet in the 3.8 miles per hour wind, however, these statistics had to be determined by difference-equation graduation, as explained by Carver (3). The analyses also appear in table 5.

Several features of these data should be discussed: (1) Even at the lowest wind velocity *Lycopodium* spores, which are heavier than the conidia of *Sclerotinia lara*, were carried upward for several feet within a comparatively short horizontal distance from the source; (2) judging from the standard deviations, vertical dispersion of the spores increased approximately in proportion to the distance from the source; (3) though the means increase slightly between 5 and 15 feet, on the whole the spore dispersion in a vertical direction occurred at random.

According to Schmidt (17) the air-borne distribution of very light seeds and of spores is normal (in a statistical sense). This conclusion follows from an analogy with the transfer of heat outward from its source. The observed distributions in table 5 partially confirm Schmidt's statement: they can be described in their gross aspects, as normal; yet, since they are rough or knobby, they cannot be regarded as coming from a normal distribution by means of random sampling alone. There is a plausible explanation: the eddies in the air move as comparatively large bodies, not as small particles. These turbulence bodies, carrying different numbers of spores and maintaining their identity for considerable periods before mixing with the surrounding air, may cause momentarily large variations in the density of spores between adjacent small areas of air. Thus in experiments such as these where the spores were released for a relatively short time, some unevenness in distributions must be expected. Extending the period of release should usually result in smoother distributions. This point will be noted again in the following section.

Mention of the size of turbulence bodies raises a question regarding the effect of tree branches on the distributions. Air movement through the orchard (without leaves, as during the blossoming period) should be more turbulent than air moving over the open ground, as in the foregoing tests. To some extent, probably, the branches would reduce the size of turbulence bodies; but in any event the mixing of the air should be increased over that occurring in the open. To determine how the tree branches affect vertical distribution of *Lycopodium* spores, four tests were conducted during the winter when trees were not in leaf. First, the spores were released over open ground and were caught 15 feet from the point of release; then the apparatus was quickly transferred to a small orchard, and the spores were so released that they were carried through the branches of one tree before being caught on the vertical series of slides 15 feet away. In all cases the air currents moved through the branches of four trees before reaching the point of release. This is mentioned because the turbulence of the air probably would increase as it passed through the limbs of more and more trees. According to table 6, the spores became more widely dispersed within the orchard than over open ground. Apparently, therefore, the vertical component of turbulence is greater in the orchard than in the open. Presumably the same would be true of the horizontal component.

The trees have an effect on wind velocity. Data on this feature are comparable, since the average wind velocity over open ground was found to vary little during the time each pair of tests (outside and inside the orchard) was conducted. The trees always reduced wind velocity, but apparently the reduction was least at the lowest velocity. When, for example, the velocities in the open were 4.9, 6.6, and 8.7 m. p. h., the velocities in the orchard decreased, respectively, 0.9, 2.1, and 3.4 m. p. h. Apparently, therefore, trees not only impede the movement of air currents but somewhat lessen the range of velocities.

TABLE 6.—*Effect of dormant orchard trees on wind velocity and on vertical dispersion of air-borne spores of Lycopodium*

Date	In open		In orchard ¹	
	Wind velocity	Standard deviation of distribution ²	Wind velocity	Standard deviation of distribution ²
	<i>M. p. h.</i>		<i>M. p. h.</i>	
Feb. 4, 1944	4.9	1.31	4.0	2.31
Jan. 27	6.6	1.78	4.5	1.99
Feb. 15	8.7	1.97	5.3	2.05

¹ A small orchard of dormant trees. In all cases the wind moved across 4 rows of trees before reaching the area where tests were conducted.

² Vertical distribution 15 feet from point at which spores were released. In the orchard the spores were released on one side of a tree and caught 15 feet away on the other side.

DISTRIBUTION OF AIR-BORNE SPORES IN THE HORIZONTAL DIRECTION TRANSVERSE TO THE MEAN WIND DIRECTION

Tracing made by means of the bidirectional wind vane show that the amplitude of the horizontal variations of eddies is greater than that of the vertical variations near the earth's surface. At a height of 2 m., for example, the ratio of the horizontal to vertical amplitudes (h/v) is 1.59. This ratio decreases to 1.2 at 18 m. and is probably 1.0 at somewhat greater heights (2). To ascertain the dispersion of spores in the horizontal direction, transverse to the direction of the mean wind, cross arms were attached (at a height of 7.5 feet) to the apparatus described earlier. Distribution of spores could then be determined for a distance of 6 feet to the right and left of the vertical support, as well as 6 feet above and below the horizontal cross arm.

Table 7 gives the results from four tests conducted at wind velocities of 3.8, 6.1, 7.2, and 10.3 miles per hour. On the whole, distribution in the horizontal direction followed that of normal probability; but difficulties were encountered in orienting the apparatus with the true direction of the mean wind. Sometimes, therefore, the peak of spore density occurred to the right or left of the center of the apparatus. When spores were released for a relatively short time, say 15 to 20 minutes, the distributions proved to be somewhat rough and knobby. The explanation, apparently, was that the shifts or variations in the horizontal direction are of fairly long duration as compared with those in the vertical direction. A common characteristic of horizontal variation seems to be progressive rather than instantaneous shifts to one side or the other, then progressive shifts in the opposite direction. As a result, clouds of spores released in rapid succession would be

carried to the right or left of the mean direction for several seconds before they are again carried the other way. When the period of release was increased to 30 minutes or more, the resulting distributions were found to resemble more nearly that of normal probability.

TABLE 7.—Comparison of the vertical and horizontal dispersion of air-borne spores of *Lycopodium* as measured by the standard deviations of corresponding distributions above and below and to the right and left of the projection of the point of release

Wind velocity (miles per hour) and dimension		Standard deviations (σ) of distributions at stated distance from point of spore release		σ 15 ft./ σ 5 ft.	σ_h/σ_v	
		5 feet	15 feet		5 feet	15 feet
3.8:	Vertical	0.62	1.59	2.56	1.74	1.56
	Horizontal	1.08	2.48	2.20		
6.1:	Vertical	.61	1.81	2.97	2.41	1.32
	Horizontal	1.47	2.39	1.63		
7.2:	Vertical	.59	1.55	2.63	1.59	1.49
	Horizontal	.94	2.32	2.47		
10.3:	Vertical	.55	1.53	2.78	1.96	1.84
	Horizontal	1.08	2.81	2.60		

The outstanding feature of the results in table 7 is the consistency with which the standard deviations of horizontal distributions exceed those of vertical distributions. At 5 feet from the point of release the ratio σ_h/σ_v averaged 1.93, and at 15 feet it averaged 1.55. The latter is very close to the ratio 1.59 obtained by means of tracings made with the bidirectional wind vane at 2 m. (6.56 feet) from the ground (2).

At 5 feet from the point of release the standard deviations of horizontal distributions averaged 1.14, at 15 feet 2.5, a ratio $\frac{(\sigma \text{ 15 feet})}{(\sigma \text{ 5 feet})}$ of 2.19. The corresponding values for vertical distribution are 0.59 and 1.62, and the ratio is 2.74. Thus the dispersion of spores in both dimensions increased roughly in proportion to distance from the source. Whether the difference between the two ratios indicates that the rate of dispersion in the vertical was somewhat greater than that in the horizontal was not apparent from the data. These results will be discussed again later in relation to wind velocity.

Whether the conidia of *Sclerotinia lara* are released into the air singly, in continuous streams, or intermittently in clouds as in the foregoing tests, probably makes little difference in the manner in which they are dispersed vertically and horizontally. When dispersion is measured in a plane perpendicular to the mean wind direction, the distribution resulting from numerous small clouds having passed through the plane resembles the distribution that would result from dispersion of individual spores in a plane through the center of a single cloud of spores moving in the direction of the mean wind. That is, spore density is greatest at the center of the plane and decreases toward the periphery. As the cloud moves farther from the source it continually expands because spores are continuously dispersed vertically and horizontally. Hence the geometrical model that best

describes the expansion of this hypothetical cloud of spores is a cone. We have just seen, however, that this cone is not circular in cross section, but is greater in the horizontal dimension. As noted earlier, though ideal conical distribution of spores does not occur in nature, the density of spores in a plane perpendicular to the projection of the apex (the point of release) varies approximately in inverse proportion to the square of the distance from the source.

According to Brunt (2), eddies of short life apparently occur at random with respect to their movement transverse to the main air current. This supposition seems to be borne out by the manner in which spores are dispersed. In order to visualize more clearly the effect of turbulence on dispersion of air-borne material, the following experiment was devised. A single vertical support bearing a cross arm 4.5 feet from the ground was constructed. Each of the vertical and horizontal arms was plainly marked at 1-foot intervals. After

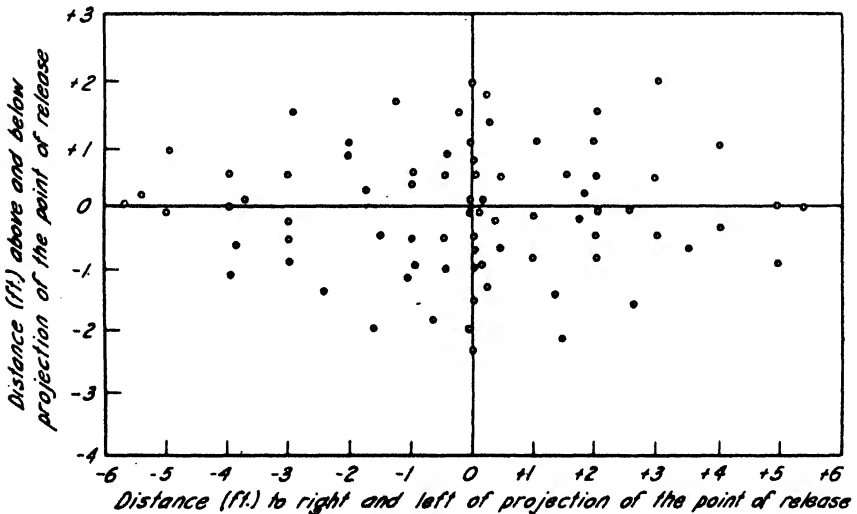


FIGURE 3.—The dispersion of "smoke" clouds by eddies in the wind. Smoke formed by aspirating fumes of ammonium hydroxide through hydrochloric acid was released from a position 4.5 feet above the ground and 16 feet from the supports. Wind velocity 6.4 miles per hour.

orienting the structure to the direction of the mean wind, small puffs of "smoke" formed by aspirating fumes of ammonium hydroxide through hydrochloric acid were released in rapid succession from a position 16 feet from the supports and 4.5 feet from the ground. As the smoke clouds passed the support, the position of their centers relative to the vertical and horizontal markings was noted, and thus data were secured from which to construct the kind of diagrams illustrated by figure 3. Though these tests were not numerous, all showed that dispersion of the individual smoke clouds was greatest at the horizontal position and decreased to a minimum at the vertical. The outline of the diagram, in consequence, was roughly oval. Though the smoke particles may not behave in the same manner as spores, the tests showed how the clouds are partitioned approximately equally between the quadrants of the oval—although, in the tests illustrated,

there was some tendency for more clouds to be carried below than above the horizontal axis, possibly because the particles were heavy enough to be influenced by gravity. What the diagram does not show is the dispersion of the particles in the individual clouds which, upon being carried downwind, rapidly expanded and reached the point of invisibility shortly after passing the support.

It is interesting to compare the horizontal and vertical dispersion of these smoke clouds with tracings obtained by means of bidirectional wind vane. The standard deviation of the horizontal dispersion (σh) in figure 3 is 2.46, that of the vertical dispersion (σv) is 0.98, and the $\sigma h/\sigma v$ ratio is 2.5. With the wind vane, tests (2) at a height of 2 feet gave an h/v ratio of 3 and at 8 feet 1.4. Interpolating to 4.5 feet, the height of the smoke tests, we obtain 2.0, a figure not far from our value.

RELATION OF WIND VELOCITY TO DISPERSION OF AIR-BORNE SPORES TRANSVERSE
TO THE MEAN WIND DIRECTION

Assuming for the moment that higher wind velocity explains why the incidence of disease decreased less rapidly with distance in 1940 than in 1939, the question is how can velocity be correlated with density of air-borne spores. One possibility would be that the spores were subject to less dispersion per unit of distance from the source in high winds than in low winds. For a given number of spores released at the source, the less the dispersion per unit of distance traveled from the source, the greater the total number carried through the recipient trees during the period of effective dissemination. We are speaking here of the total numbers of spores reaching equal areas in the recipient trees at different distances from the source, and not of the rate at which these numbers decrease, for, in the experimental results presented earlier, the density of air-borne spores decreased at the same rate at all wind velocities between 2 and 16 miles per hour. Since the spore dispersion follows an approximate conical pattern, and since in the experiments relating to spore density the samples collected by means of oil-coated slides were uniformly scattered over right sections of cones at different distances from the apex, then for each apex distance we were dealing with a constant proportion of the total number of spores liberated. This is so because the area of the cone base is proportional to the square of the distance from the apex. Besides, as shown earlier, the standard deviations of spore distributions, which express the degrees of dispersion, are also proportional to the distance from the apex; hence the area of the right section is proportional to the square of the standard deviation. When the ratio of the number of spores per unit area (density) is taken for two different distances, the total number of spores and the constant proportion of this total number, which depend upon the degree of dispersion, cancel out. The result; that is, the rate of changes in density—is then independent of the degree of dispersion, and therefore of any effect that wind velocity might have on dispersion.

If, on the other hand, the rate at which the spores disperse as they are blown downwind is altered, the rate at which their density decreases from one distance to another will not change; but the total number of spores passing through a given area of a vertical plane will. A decrease in dispersion rate can be visualized as a contraction in the

diameter of the hypothetical cone of dispersion. Thus, with a given number of spores liberated at the source, the more the cone contracts the greater is the number of these bodies passing through a unit area of its right sections.

Effects of wind velocity must be looked for, accordingly, in the data on spore dispersion, not in the data on comparative densities. Aside from somewhat larger standard deviations for distributions at 3.8 miles per hour, the values pertaining to dispersion in table 5 change little if any with changes in wind velocity. Those in table 6 increased somewhat, whereas those pertaining to vertical dispersion in table 7 decreased slightly. In another series of 26 tests, vertical dispersion was determined at 5 feet from the source. The mean values presented in table 8 indicate that dispersion was high at low velocities but varied little between medium and high velocities. These data and those in table 5 are open to two interpretations: (1) The degree of dispersion decreases rapidly between low velocities, but much less rapidly between medium and high ones; (2) if velocities (5 miles per hour or lower) at which winds are notably variable in direction are disregarded, the degree of dispersion at equivalent distances displays no significant tendency to change with wind velocity.

TABLE 8.—Standard deviations of vertical distributions of air-borne spores of *Lycopodium* 5 feet from the source at various wind velocities

Wind velocity (miles per hour)	Mean standard deviations (σ) of vertical distributions	Wind velocity (miles per hour)	Mean standard deviations (σ) of vertical distributions
1-3	1.57	9-11	.45
3-5	1.13	13-15	.62
5-7	.58	15-17	.59
7-9	.64	17-19	.45

Presumably dispersion would be affected by changes in the turbulence of the air. At the height at which these tests were conducted (7.5 feet) the main source of turbulence is friction along the earth's surface (15). A second source, thermal, is afforded by a decrease in air temperature with height above the earth. If this decrease is greater than the adiabatic lapse rate, warm air will tend to rise. On the whole, thermal turbulence is most pronounced at low wind velocity. Even moderate air flow prevents the formation of a steep temperature gradient and thus is unfavorable for thermal turbulence. Frictional turbulence, on the other hand, increases with increased air flow. Gustiness of the wind, and consequently the strength of cross eddies, is said (15) to be about proportional to wind velocity. Judging from measurements made with the bidirectional wind vane, the ratio of vertical to downwind velocities in eddies increases with wind velocity, but the ratio of the horizontal to downwind velocities is less noticeably affected (2). The ability of strong winds to lift and carry dusts to great heights is widely recognized. Apparently less is known, however, about the effects of variations in wind velocity on the rate at which wind-borne material is elevated in comparison with the rate at which it is carried downwind. Consider, for example, the situation illustrated by figure 4. Assume that dispersion at a distance

x for an 8 miles per hour wind is 1. Assume also that as wind velocity increases the rate of turbulence increases, but somewhat more slowly than that of wind movement. If an 8 miles per hour wind moves a distance x in t time, a 16 miles per hour wind would move the distance x in $\frac{1}{2} t$ time or $2x$ distance in t time. In table 5 we can compare the standard deviation (0.73) of dispersion at 5 feet from the source for an 8.1 miles per hour wind with that (1.37) at 10 feet from the source for a 16 miles per hour wind; when velocity doubles the degree of dispersion increased 1.9 times.

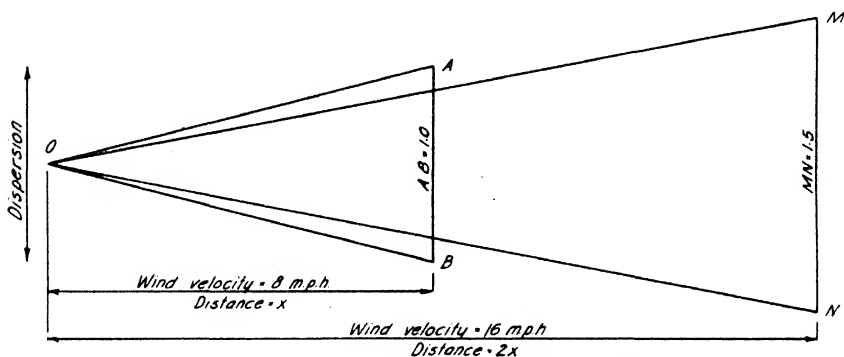


FIGURE 4.—A comparison of the relations between the degree of dispersion of air-borne spores at a given distance, x , from the source of spores when the rates of increase in dispersion and wind velocity are 1.5 and 2, respectively.

However, to show wind-velocity effects in figure 4 the degree of dispersion for a 16-mile-per-hour wind is assumed to be 1.5 at $2x$ distance. Representing the dispersion in an 8-mile-per-hour wind by bar AB at x distance from the source O , and dispersion in a 16-mile-per-hour wind by bar MN at $2x$ distance, if dispersion is conical; that is, if it increases in proportion to the distance from the source, as indicated by the experimental results—the degree of dispersion at all distances from the source will be represented by lines OA and OB for an 8-mile-per-hour wind, and by lines OM and ON for a 16-mile-per-hour wind. In this situation, as inspection reveals, the degree to which the spores are dispersed at x distance by a 16-mile-per-hour wind is less than that for an 8-mile-per-hour wind. At a given distance, therefore, the degree of dispersion will diminish as wind velocity rises, unless the increase in the rate at which the spores are dispersed equals or exceeds the increase in the rate at which they are carried downwind.

With these relations in mind we shall reexamine the spore-dissemination results. At low-wind velocities dispersion was frequently much greater than at medium velocities; but it did not diminish noticeably throughout medium and high ranges (tables 5 and 8). Many of these tests were conducted on sunny days when conditions favored thermal turbulence. This type of turbulence probably played a considerable part in vertical dispersion, especially when wind movement was low. Moreover, because winds of low velocity are noticeably variable in direction, the spores would probably be much more widely scattered at such velocities than at higher ones. For these reasons, we are not fully warranted in attributing the rapid decrease in dispersion between low and medium velocities to the diminishing effects of

frictional turbulence. If results obtained at velocities below 5 miles per hour are excluded, the standard deviations obtained for medium and high velocities are found to exhibit no consistent variations. For example, none of the values for velocities above 3.8 miles per hour in table 5 show marked differences, especially if corresponding standard deviations for 5.9 and 6.4 miles per hour and those for 8.1 and 8.5 miles per hour are averaged. Likewise, the values for velocities above 5 miles per hour in table 8 show little tendency to vary with wind velocity. If, therefore, the interpretation regarding the relation of dispersion to distance, as given in figure 4, is correct, these results indicate that dispersion increases at approximately the same rate as wind velocity for distances up to 15 feet. The standard deviations in table 6, column 3, indicate, on the other hand, that the rate of dispersion increased about 1.37 times faster than wind velocity, whereas the values pertaining to vertical dispersion in table 7 indicate the rate of increase to be about 0.85 times that of the increase in wind velocity. Thus the average value for all four series is approximately 1—a unit change in velocity resulted in a unit change in vertical dispersion.

About horizontal dispersion much less is known. Judging from the results reported for the bidirectional wind vane (2), turbulence in this dimension changes little if any as wind velocity increases. If such is the case, we should expect from the analysis in figure 4 that standard deviations of horizontal distribution at 5 and 15 feet in table 7 would decrease as velocity increased from 3.8 to 10.3 miles per hour. There is a suggestion of a decrease; but, as noted earlier, considerable difficulty was encountered in determining over-all distribution in the horizontal, and so the results do not justify conclusions.

Turning to the results on spread of the disease in apricot orchards (table 1), we find the incidence of blossom infection between the source and the fourth row of recipient trees (88 feet) to have decreased 94.5 percent in 1939 and 77 percent in 1940, a difference of 17.5 percent. Presumably, therefore, the density of spores varied to a similar degree. A difference of 17.5 percent in dispersion at 88 feet from the source probably represents a much smaller difference in density. In any event, these results may be explained as due to the differences between wind velocities at the two effective dissemination periods, provided the rate at which the spores were dispersed relative to the rate they were carried downwind is assumed to have been less in 1940 than in 1939. In some of the tests mentioned above, the ratio of change in wind velocity to change in vertical dispersion was 1.0 : 0.85; but this may have been due to chance, because in other tests the ratio was 1.0 : 1.37 and the average of all tests was approximately 1.0 : 1.0. If increases in horizontal dispersion did not keep pace with increases in wind velocity, the aerial density of the spores would diminish as wind movement increased in cases where the source of spores is a limited area. When, however, the source extends an appreciable distance transverse to the wind direction, such as a block of trees may do, dispersion in the horizontal would probably not be greatly affected. Apparently, therefore, the assumption above will be valid only if the rate of vertical dispersion increases somewhat more slowly than that of wind velocity.

INTERCEPTION OF SPORES BY THE TREES AS A FACTOR IN AERIAL DENSITY AT INCREASING DISTANCES FROM THE SOURCE

What effect does the interception of air-borne spores by the trees have on the density of these bodies? This discussion was purposely delayed until all experimental data had been presented. More specifically the question is, will the number of spores caught by the trees significantly diminish the density at subsequent distances, and might the number caught vary with wind velocity?

That such a loss reduces the aerial density cannot be denied. As will be recalled, however, in the experimental work the glass slides caught large numbers and yet their aerial density did not consistently diminish more rapidly than was to be expected from conical dispersion. In these tests, moreover, the decrease in density was not affected by variation in wind velocity. The number of spores caught on branches and blossoms may have affected the incidence of disease in recipient trees, but how greatly cannot be estimated.

MATHEMATICAL MODEL OF DISPERSION IN WIND-BORNE SPORES

Despite some conflicting results reported in the previous sections, certain features seem to be clearly established. We shall list them.

1. The average wind velocity in 1940 was 1.88 times greater than that of 1939 during the respective periods of effective spore dissemination.
2. The horizontal and vertical distributions of spores were approximately normal.
3. The results obtained in the open are not directly applicable to orchard conditions; for instance, the wind velocity is reduced 18, 32, and 37 percent for velocities of 4.9, 6.6, and 8.7 miles per hour (table 6), and the limbs have a pronounced effect on turbulence.
4. The standard deviations of the vertical and horizontal spore distributions increase with distance from the source.
5. The infection gradients were very different in the 2 years 1939 and 1940. It is not definitely established that wind velocity affected the standard deviations of the spore distribution at equivalent distances from the source. Certain of the experimental data suggests, however, that this might be the case under some conditions.

We shall construct a mathematical model of wind-borne spore dispersion that conforms to the clearly established facts. Some gaps will have to be bridged. If further investigations show that this bridging has not been done properly, then the model can be modified to take account of the new data.

First let us observe that the data of figure 5 can be very well represented by the equations

$$y = \frac{1,938}{x^{1.2362}} \quad (1)$$

for the year 1939 and

$$y = \frac{551.8}{x^{0.7123}} \quad (2)$$

for the year 1940, where y is the percent of infection in successive rows of recipient trees in terms of the percent of infection in the source trees and x is the distance of the centers of successive recipient

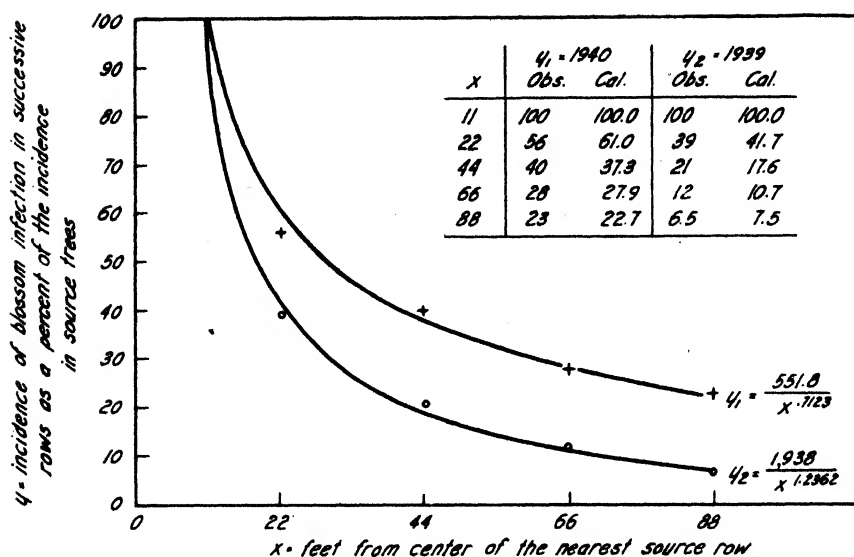


FIGURE 5.—The relative incidence of blossom infection by *Sclerotinia lara* in apricot trees at various distances from the source of conidial inoculum, and at mean wind velocities of 15 miles per hour (+) in 1940 and 8 miles per hour (°) in 1939. The curves were fitted by means of equations developed from a theoretical treatment of spore distributions given in the following section.

rows from the center of the first row of the source trees—that is, x takes the values 22, 44, 66, 88 for the successive recipient rows (table 1). These formulas⁷ were obtained by making an equation of the form

$$y = \frac{A}{x^p} \quad (3)$$

pass through the point with an abscissa of 11 and an ordinate of 100, and then averaging the observational equations to obtain the value of $\log A$. The abscissa value of 11 feet seems to be indicated for the following reason: Since the branches of the trees extended approximately halfway between rows, the area from which the spores were supplied did not end at the center (trunks) of source trees that were nearest the first row of recipient trees. A distance halfway (11 feet) between the last source row and the first recipient row, therefore, was chosen as the place at which a decrease in the aerial density of the conidia began. Choosing this point for the beginning of decrease in infection permits very good representations of the observed data as shown in figure 5.

Let us now begin at the beginning of spore dispersion and see what is necessary to arrive at the result expressed by equation 3. According to Brunt (2), who was reporting the work of others, the

⁷An article by P. H. Gregory (5) was published in the Transactions of the British Mycological Society after the present paper was submitted for publication. To describe the pattern of spore dispersion Gregory employs an equation developed by meteorologists from studies of the transfer of heat, momentum, and matter by eddy diffusion. In general, this equation and equation 3 of the present paper give similar results, the major difference being in the range of the exponent for distance, x .

standard deviations of horizontal and vertical distributions of smoke particles, dye in water, and the like are of the form

$$\sigma = ax^p \quad (4)$$

Here σ is the standard deviation of a distribution at a distance x from the source, a is a constant, and p is a number between $\frac{1}{2}$ and 1; and the horizontal distributions are independent of the vertical distributions.

In view of the investigations carried out by others (8) as well as those cited in this paper we shall assume that

$$\sigma_{vx} = ax^p, \quad \sigma_{hx} = bx^q \quad (5)$$

Here σ_{vx} and σ_{hx} are respectively the standard deviations of vertical and horizontal spore distributions at distance x from the source; a , b , p , and q are quantities that may depend on wind velocity but do not depend on x ; and the horizontal and vertical distributions are normal and independent of each other. We shall admit values from $\frac{1}{2}$ to 2 for p and q , instead of $\frac{1}{2}$ to 1 as other investigators have suggested. We must assume that p , q , a , and b depend on wind velocity, in order to account for the observed difference between infection gradients for the 2 years 1939 and 1940. Separate representations are used for σ_{vx} and σ_{hx} because, as shown in the previous discussions, the vertical and horizontal distributions behave somewhat differently.

On the basis of these assumptions and the consideration of a block of sources, we are led to equation 3 as a valid approximation of the infection gradient if x is sufficiently large.

If we plot our two values of p given in equations 1 and 2 against wind velocity and extrapolate back to zero wind velocity, we get 1.81 for p . This is fairly close to 2.0, the value of p corresponding to the relation between y and x for a conical distribution. Judging from the present data, p may lie between 0.5 and 2.0. We are now considering a block source, not a point source as in the experiments described in the earlier sections.

The percentages of infection, furthermore, depend only on the standard deviations of the vertical distributions.

In conclusion, let us make three assumptions—(1) the percent of infection is proportional to the number of spores reaching a given area; (2) the general level of blossom susceptibility is measured by the percent of blossom infection in the source trees; (3) spores fan out from a single source in such a way that both horizontal and vertical distributions are normal and independent, with standard deviations proportional to some power of the distance from the source (not necessarily the same) and with means that lie on the line of projection of the source in the average direction of the wind. Then the ratio, y , of the percent of infection in a vertical slice of susceptible blossoms at a perpendicular distance x from the source plane to the percent of infection in the source trees is given to a good first approximation by an equation of the form

$$y = \frac{A}{x^p}$$

Here A and p are constants that depend on average wind velocity and perhaps, to a lesser extent, on many other quantities; and x is

measured from the center of the first row of source trees. The assumptions stated above have been made plausible by data collected in specially designed experiments and by a wide survey of the existing literature. Two distinct sets of data have been successfully treated on the basis of these assumptions (fig. 5).

DISCUSSION

Many details concerning the aerial dissemination of spores and the spread of the disease that the spores incite must be studied before general conclusions are warranted. Further study of certain details, however, must await an improvement in the method for measuring dispersion, since the method for ascertaining the over-all distribution of spores at intercepting planes is not adapted to greater distances than those employed herein. Additional studies should include, among other things, a comparison of dissemination over open ground with that through orchard trees.

Since the zone of frictional turbulence in the atmosphere is limited in depth and since the magnitude of its vertical component varies with height, the pattern of spore dispersion aloft might well differ from that near the earth's surface. Judging from results by Durham (12), spores carried into the upper air may at times reach a "ceiling" above which they are not readily dispersed. Such a ceiling might well occur at the lower boundary of an inversion in the upper atmosphere. According to Lyman (10), the smoke from certain forest fires remained in the air at a density sufficient to affect visibility for hundreds of miles southeastward. Points regarding the long-distance transportation of spores in air masses are discussed by Durham (12), Lambert (9), Rittenberg (16), ZoBell (12), and Jacobs (7).

SUMMARY AND CONCLUSIONS

Conidia of *Sclerotinia laxa*, the cause of a blossom blighting of stone-fruit trees, are produced in large numbers during late winter on blossoms and twigs that were blighted the previous spring. These are detached from the sporophores by winds of low velocity and, judging from their rate of fall in still air, are readily transported by air currents.

Spread of the disease through apricot orchards by means of air-borne conidia northward from well-defined sources of spores have been observed. For three disease gradients studied in 1939 the mean levels of blossom infection in trees 22, 44, 66, and 88 feet from the nearest source trees were 39, 21, 12, and 6.5 percent, respectively, of the level in source trees. For four gradients studied in 1940 the corresponding values were 55.5, 40, 28, and 23.

The mathematical treatment of these data minimized or eliminated the effects of such factors as the number of spores produced and liberated at the source, the length of the dissemination periods, and variations in the environmental conditions that initiate infection. For this reason, an explanation of the difference between the 1939 and 1940 gradients was sought elsewhere. According to a study of the circumstances preceding blossom infection, in 1939 and again in 1940 there was only one period at the susceptible (blossoming) stage of the host when the direction of the wind favored spore dissemination northward from the source. During the 1940 period mean wind velocities

were 1.88 times higher than during the 1939 period. The amount of blossom infection was assumed to be proportional to the number of spores reaching the blossoms, and the unilateral characteristics of the gradients were judged to be expressions of variations in the aerial density of spores during the effective dissemination periods. Factors capable of disturbing the relations between incidence of the disease and aerial density of spores are discussed.

Variations in aerial density of spores as distance from the source of spores increased were studied by releasing *Lycopodium* spores and catching them on glass slides placed 5, 10, and 15 feet downwind from the point of release. The density—that is, the number of spores passing through a unit area of intercepting planes—varied approximately in inverse proportion to the square of the distance from the source, a relation to be expected if the dispersion of the spores is described by a horizontal cone having its apex at the source of spores and its base oriented to the direction of the mean wind.

According to other tests the over-all distribution of these spores along the vertical and horizontal axes of intercepting planes followed roughly that of normal probability; that is, the density of these bodies was greatest in the center of the plane (at the level of release) and decreased progressively above and below and to the right and left of the center. The standard deviations of the distributions were employed, therefore, to express the degree of dispersion at various distances from the source. These values increased approximately in proportion to the distance from the source. At equivalent distances, however, the standard deviation for horizontal distributions (σ_h) were consistently greater than those for vertical distributions (σ_v). When spores were released 7.5 feet from the ground, the mean σ_h/σ_v ratio was found to be 1.55, a value very close to 1.59, the figure obtained by others in measuring the amplitude of the horizontal and vertical components of turbulence by means of the bidirectional wind vane placed 6.56 feet from the ground. Studies of the dispersion of small puffs of ammonium chloride "smoke" gave similar results.

The effect of trees (without leaves) on vertical dispersion of spores was studied.

The rate at which aerial density of spores decreased with increases in distance from the source was apparently not affected by variations in wind velocity between 2 and 16 miles per hour. For winds of low velocity (below about 5 miles per hour), however, dispersion at a given distance from the source was often greater than that for medium and high velocity. Between medium and high velocities, on the other hand, dispersion varied little within the distances studied (5 to 15 feet).

Since frictional turbulence increases with wind velocity, aerial dispersion of spores should likewise increase, other things being equal. The degree of dispersion at a given distance from the source, however, is determined by the rate the spores are dispersed in relation to the rate they are carried downwind. When the source is a limited area at least, the degree of spore dispersion at a given distance will probably decrease as wind velocity increases—unless the effects of turbulence on dispersion increase as fast as wind velocity, or faster. On the whole, the experimental results indicate that dispersion increased at about the same rate as velocity, except at velocities below about 5 miles per hour; but further study of this point is desirable. In par-

ticular, dissemination should be studied at distances from the source greater than those employed herein.

To compare the results from observations on disease spread with those from the experiments, the latter were generalized in terms of dissemination (1) from a three-dimensional block source instead of from a "point" source, (2) through orchard trees instead of over open ground, and (3) for distances up to 100 feet instead of 15 feet. Certain gaps were bridged by assumptions, which, if rendered untenable by further studies, can be modified to conform with the new information. Though the results of this treatment must remain provisional, the observed spread of the disease through the apricot orchards is described to a good first approximation by an equation of the form $y = \frac{A}{x^p}$, where y is the ratio of the percentage of blossom infection in a vertical slice of susceptible tissue (blossom) at a horizontal distance from the source block to the percentage of blossom infection in the source trees, A and p are constants depending on wind velocity and perhaps on other quantities to a lesser extent, and x is the horizontal distance from the center of the nearest source trees.

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SUSCEPTIBILITY OF HIBERNATING CODLING MOTH LARVAE TO LOW TEMPERATURES, AND THE BOUND-WATER CONTENT¹

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INTRODUCTION

The codling moth (*Carpocapsa pomonella* (L.)) is a pest of apples wherever they are grown in the United States. It is able to survive under a diversity of climatic conditions, prominent among which are low winter temperatures. At times, however, overwintering larvae are killed by exposure to low temperatures. At Yakima, Wash., for example, Newcomer (5)³ found that wherever the minimum temperature had been lower than -25° F. all overwintering larvae with no protection other than bark or burlap bands were killed.

A study was therefore undertaken to find out what low temperatures are fatal to larvae of the codling moth, whether larvae indigenous to widely separated apple-growing districts show any differences in susceptibility to cold, and whether cold-hardiness is influenced by differences in sex or weight of the larvae or by differences in humidity of the atmosphere before exposure to cold. Determinations were also made of the bound-water content of hibernating codling moth larvae, since bound water is generally believed to have a part in cold-hardiness.⁴

THE FREEZING OF INSECTS

Some insects can be cooled considerably below their true freezing temperature before actually freezing. The point reached just as freezing commences is referred to as the undercooling temperature. When the first ice crystal is formed, the heat of crystallization causes a sudden rise, the limit of which is called the rebound temperature. The temperature of the insect then remains constant for a moment, until the body fluids solidify, and then falls again to the temperature

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³ Italic numbers in parentheses refer to Literature Cited, p.340.

⁴ Because of space limitations many details of procedure, the formulas used for computations, and certain data have been omitted. Any desired information on these details will be furnished by the author on request.

of the surrounding media. The true freezing temperature of the insect is somewhat higher than the rebound temperature, because part of the heat of crystallization is transferred to the surrounding media. No attempt has been made in this study to determine the true freezing temperature of the codling moth. A typical cooling curve for the codling moth is shown in figure 1.

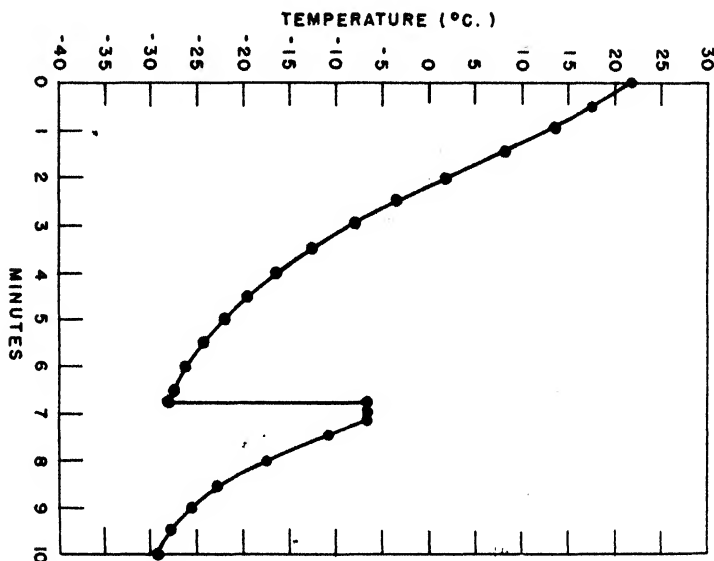


FIGURE 1.—Typical cooling curve for the codling moth larva, showing undercooling and rebound temperatures.

Several theories have been advanced to explain the part freezing plays in killing insects. An early view that the formation of ice in the cells causes death through mechanical injury has largely been abandoned. Some investigators believe that withdrawal of water from the cells is responsible, others that the normal functionings of the cell walls are destroyed.

Bachmetjew (1), one of the first entomologists to study the cold-hardiness of insects, reported that death occurred only after the insect had been subjected to its undercooling temperature a second time. As found by the author, and as pointed out by Salt (11) and others, some insects can survive freezing, and are killed only by temperatures below their undercooling point.

Robinson (8, 9) studied the possible correlation between the hydrophilic colloid content of an insect and its winter-hardiness. Some investigators believe that such colloids adsorb sufficient water to lower the fatal temperature. This adsorbed water is called bound water, whereas the other water in the insect is known as free water. The properties of water associated with colloids are known to differ from those of true solutions. Thönius (13) stated that bound water will not freeze at $-20^{\circ}\text{C}.$, and Jones and Gortner (3, p. 434), using the dilatometric method, found that the bound water present in

gelatin and the white of egg did not freeze even at -50°C . These authors state:

It is emphasized that "bound" water is an indeterminate term, and that "bound" water values as experimentally determined may be expected to vary from system to system, the variation being due to many factors, not the least of which is the method selected for measurement. If biological cells and tissues are similar in their behavior to gelatin and (probably) to the thick portion of egg white, then "bound" water is a measurable entity and (using dilatometric procedure) has a constant value at least at temperatures between -6° and -50°C .

On the basis of these theories Robinson (8, 9, 10) made studies of the proportion of bound water present in several species of insects. Kistler (4), however, has questioned the validity of results based on these hypotheses, on the ground that the undercooling of some of the water would interfere with their accuracy. More recently Ditman, Vogt, and Smith (2, p. 272) have expressed the opinion "that unfreezable water determinations are of no value in estimating the relative cold-hardiness of insects."

STUDIES OF UNDERCOOLING AND REBOUND TEMPERATURES

COLLECTION OF LARVAE

Representative overwintering larvae from different sections of the United States were obtained by banding apple trees in fruit districts in five States, as follows: Maryland (Easton), Georgia (Cornelia), Arkansas (Bentonville), Colorado (Grand Junction), and Washington (Yakima). The bands containing the larvae were shipped to Takoma Park, Md., where they were held in an outdoor insectary until used in these studies.

APPARATUS AND PROCEDURE

The apparatus for determining the undercooling and rebound temperatures consisted essentially of a refrigerator for cooling the larvae and a temperature-recording device (fig. 2).

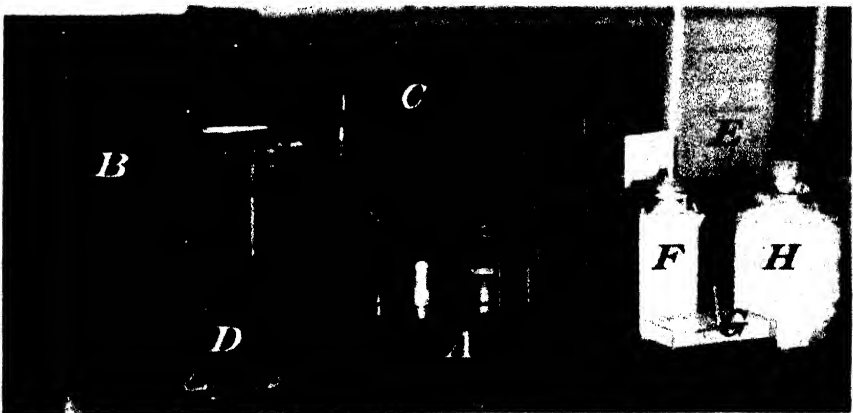


FIGURE 2.—Apparatus used in determining undercooling and rebound temperatures of codling moth larvae: A, Potentiometer; B, hood for galvanometer (not visible) and scale; C, scale for galvanometer reading; D, standard cell; E, battery; F, cold junction; G, mercury switch; H, vacuum-bottle refrigerator.

The refrigerator (*H*) was a wide-mouth vacuum bottle supported within a cotton-filled battery jar. A cell (fig. 3) for holding the insect was made out of two corks, one larger than the other. The smaller end of one cork was hollowed out enough to hold the insect and inserted in a pocket in the larger cork which had been cut out and filled with cotton. Thermocouple wires were inserted through the smaller cork so that the junction would be in contact with the larva. The cell was then placed in a container consisting of two test tubes one inside the

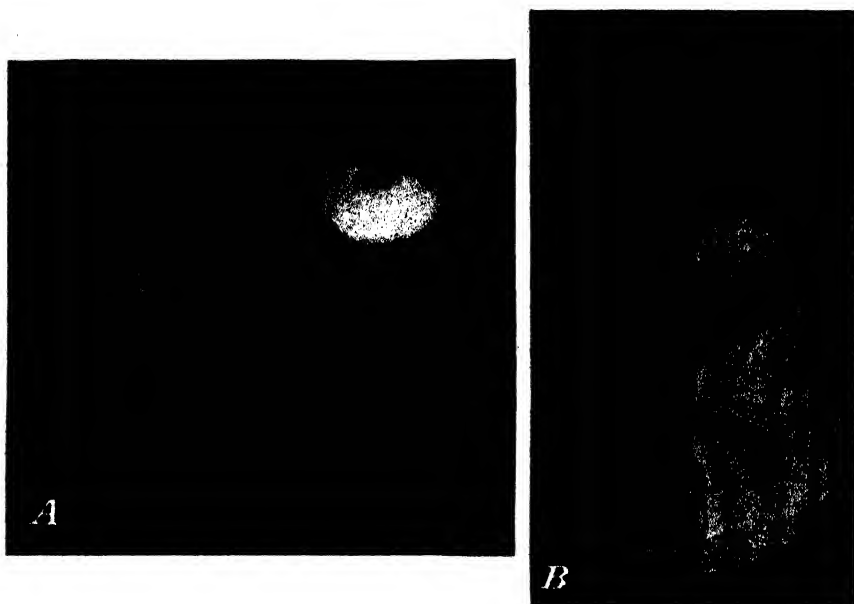


FIGURE 3.—Insect cell for determination of undercooling and rebound temperatures of the codling moth larva: *A*, Cell open showing position of larva and thermocouple wires; *B*, cell closed.

other, and inserted in the vacuum bottle through a hole in its cork stopper.

To produce low temperatures a eutectic mixture of calcium chloride hexahydrate and finely crushed ice or a mixture of solid carbon dioxide and gasoline was used. The lowest temperature obtained with the first mixture was -37°C ., although it is capable of giving -54.9° .

All temperature measurements were made with a potentiometer (fig. 2, *A*) in connection with a D'Arsonval galvanometer and a thermocouple of copper-constantan wire. The cold-junction ends of the thermocouple were lowered into small glass tubes containing a half-inch of mercury, which were inserted through a cork stopper into a pint vacuum jar filled with finely crushed ice (*F*). To reduce electrical resistance a home-made mercury-pocket reversing switch (*G*) was employed in place of a knife switch. The thermocouple was calibrated as to electromotive force-temperature function in accordance with a standard equation and constants.

EXPERIMENTAL RESULTS

In the tests reported in this paper no codling moth larvae survived after one exposure to their undercooling temperature, although a few individuals lived in a very weak condition for several weeks following their freezing.

EFFECT OF LOCALITY, SEX, AND WEIGHT

The undercooling and rebound temperatures of larvae from several fruit districts in the United States are summarized in table 1. Practically no differences due to locality of origin are indicated by these data.

TABLE 1.—*Relation of locality of origin of codling moth larvae to their undercooling and rebound temperatures, 1931*

State	Larvae	Mean under-cooling temperature	Mean rebound temperature
	Number	° C.	° C.
Maryland.....	3	-26.0±1.4	-6.0±0.4
Colorado.....	11	-25.9±.7	-6.2±.5
Arkansas.....	5	-27.0±.6	-7.4±.3
Georgia.....	21	-25.8±.6	-6.7±.6
Washington.....	23	-26.0±.6	-6.9±.8

Similar data for other groups of larvae separated according to sex are given in tables 2 and 3.

TABLE 2.—*Relation of locality of origin, sex, and weight of codling moth larvae to their undercooling temperatures, 1932*

State	Sex of larvae	Number of larvae	Mean weight	Mean under-cooling temperature
			Gram	° C.
Georgia.....	{ Male.....	6	0.039	-28.3±0.6
	{ Female.....	9	.047	-25.5±1.6
Maryland.....	{ Male.....	5	.039	-26.4±1.8
	{ Female.....	10	.050	-25.6±.6
Washington.....	{ Male.....	9	.039	-26.1±.5
	{ Female.....	11	.052	-27.2±.3

TABLE 3.—*Relation of locality of origin, sex, and weight of codling moth larvae to their undercooling and rebound temperatures, 1933*

State	Sex of larvae	Number of larvae	Mean weight	Mean under-cooling temperature	Mean rebound temperature
			Gram	° C.	° C.
Maryland.....	{ Male.....	3	0.054	-27.7±0.9	-8.8±1.1
	{ Female.....	1	.059	-28.3	-5.0
Colorado.....	{ Male.....	6	.035	-29.4±.7	-11.1±1.3
	{ Female.....	5	.041	-29.3±.5	-11.2±1.2
Arkansas.....	{ Male.....	2	.041	-28.4±1.7	-8.0±.8
	{ Female.....	2	.046	-28.9±1.0	-9.5±.8
Georgia.....	{ Male.....	4	.047	-30.1±.7	-9.3±2.0
	{ Female.....	7	.055	-27.3±1.0	-6.5±.7
Washington.....	{ Male.....	2	.041	-24.4±1.6	-7.2±2.1
	{ Female.....	2	.042	-26.5±3.0	-9.7±1.7

The results presented indicate that neither the undercooling nor the rebound temperature is appreciably influenced by the locality from which the insects are obtained or by the weight or sex of the larvae. These conclusions are supported by other work, presented in the next section, although the data for individual localities are not given.

EFFECT OF DRY AND HUMID CONDITIONS

Payne (6), in experiments with *Synchroa* larvae, demonstrated that both the undercooling and the freezing temperature could be lowered by dehydrating the larvae. This was true whether or not the insects were normally self-dehydrating. Payne also found (7) that the larva of the Japanese beetle (*Popillia japonica* Newm.), which normally overwinters in a moist environment, is very resistant to cold when dehydrated to half its body weight.

A series of tests was conducted in which larvae, undisturbed in their cocoons, were held at 10° C. in sealed glass containers for 1 to 4 months, some under humid and others under dry conditions, before their undercooling and rebound temperatures were taken. Moisture was supplied by means of lampwicks dipped into a beaker of water. For tests under dry conditions the air in the container was dehydrated with silica gel, which was thoroughly dried out weekly. The exact extent of the dehydration was not determined for these larvae. Because of the codling moth larva's habit of spinning new cocoons if disturbed, it was not possible to weigh naked larvae before and after dehydration without affecting the normal condition of the insect.

The summarized results, given in table 4, indicate that the average undercooling and rebound temperatures of the insects were not markedly affected by the extremes in humidity under which the larvae had been kept. It is believed, therefore, that the resistance of the dormant codling moth larva to low temperatures is not greatly changed by wide differences in humidity such as sometimes exist under field conditions.

TABLE 4.—Undercooling and rebound temperatures of hibernating codling moth larvae that had been subjected to dry and humid conditions previous to exposure to low temperatures

Atmospheric condition previous to test	Sex of larvae	Number of larvae	Average weight	Mean under- cooling tem- perature	Mean re- bound tem- perature
			Gram	° C.	° C.
Dry	{ Male	9	0.036	-25.6	-8.3
	{ Female	16	.051	-26.8	-7.5
Humid	{ Male	13	.044	-25.7	-7.1
	{ Female	12	.053	-27.7	-7.9

EFFECT OF CROSS-BREEDING

To determine the effect of cross-breeding on resistance to low temperatures, male moths from Washington were crossed with females from Georgia. As far as could be determined from five crossbred males, and six crossbred females, crossing produced no change in the susceptibility of the resulting larvae to low temperatures.

PROTECTIVE VALUE OF UNDERCOOLING

As the temperature falls, the larva lies quiet in its cocoon, and its metabolic processes become for the most part inactivated. This state of rest perhaps favors undercooling in the same way that quiet conditions permit water to be undercooled considerably below 0°C . There is no chance for foreign substances to be introduced into the body fluids of the larva to serve as nuclei for ice formation. Moreover, the host tree, on which the larva is quartered beneath the bark, is relatively free from vibrations.

Since the temperature required to produce the first crystal of ice is much lower than the true freezing temperature, the ability to undercool permits the insect to withstand winter temperatures many degrees below its true freezing temperature.

To determine whether the inactivity of the larvae during the winter facilitates undercooling, tests were conducted with three larvae from Maryland and four from Arkansas. As the temperature was lowered, the larvae were kept in motion by means of a wire looped around their thoracic segments, while the thermocouple remained in contact with the abdominal segments. Both the undercooling and the rebound temperatures were considerably higher than when the larvae were undisturbed. The fact that undercooling occurred at all is evidence that the tendency to undercool is exceedingly pronounced.

DETERMINATIONS OF BOUND WATER

Bound-water determinations were made by two methods. The first was the dilatometric method, which had been used by Jones and Gortner (3) for various gels and by Sayre (12) for plant tissue. The second method was the heat-of-fusion-of-ice method, which had been used by Robinson (10) and others.

DILATOMETRIC METHOD

The dilatometric method takes advantage of the expansion that occurs with the freezing of water to measure the quantity of water that freezes in the larvae. The total water content is then determined by drying the larvae, and the bound water is determined by difference.

The dilatometer used (figs. 4 and 5) consisted of a graduated capillary tube of 1-mm. bore and 55 cm. long fused to a 25-ml. bulb into which was ground a glass stopper provided with ears for the attachment of rubber bands. Toluene was selected as a suitable liquid, since it is practically insoluble in water and has a low freezing point. Two glass containers, one inside the other, separated by 3 inches of ground cork formed an insulated chamber, which was filled with calcium chloride solution. This chamber was equipped with a motor-driven stirrer.

Approximately 125 codling moth larvae, having a total weight of about 5 gm., were used in each test. After being weighed they were introduced into the dilatometer bulb containing toluene. Additional toluene was then drawn in through the capillary tube with an aspirator to a certain scale reading, while the glass stopper, smeared

with glycerol, was slowly revolved until it seated, care being taken to exclude air bubbles. As extra precautions rubber bands were used and the stopper was sealed with celluloid cement.

The calcium chloride bath was cooled to and kept constant at -7.5°C . This temperature was selected because it is between the maximum undercooling temperature and the freezing temperature of the larvae.

The dilatometer containing the larvae was then placed in the bath, and when thermal equilibrium was established the reading was taken. The dilatometer was next transferred to a freezing chamber at -35°C . and held therein until all the larvae were frozen, when it was replaced in the bath at -7.5°C . The quantity of water that was frozen within the larvae was computed from the difference in the readings of the dilatometer when the larvae were in the unfrozen and the frozen state. The larvae were then removed from the dilatometer and placed in an electric oven at $87^{\circ}\text{--}89^{\circ}\text{C}$., where they were desiccated under vacuum until a constant weight was obtained. The total quantity of water originally present in the larvae was then calculated. From

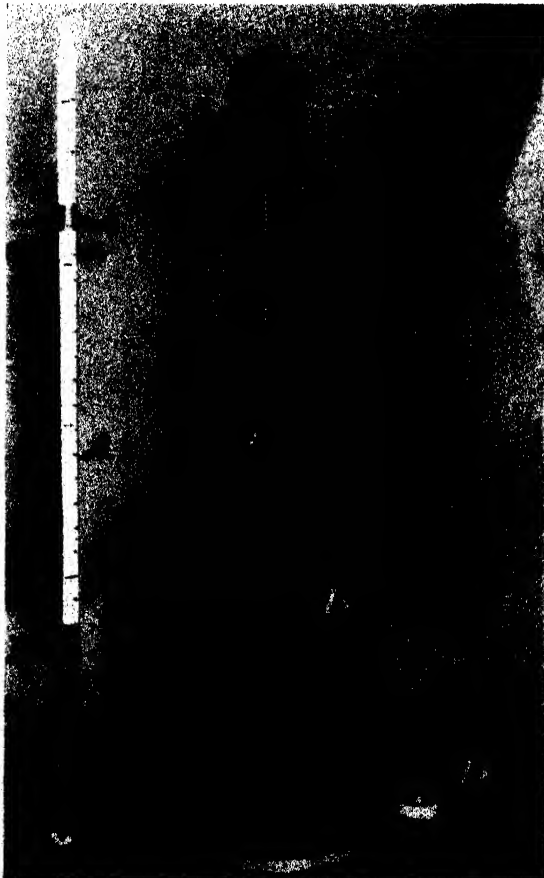


FIGURE 4.—*A*, Dilatometer; *B*, insulated temperature chamber; *C*, stirrer; *D*, rheostat; *E*, thermocouple.

these two figures was computed the percentage of the total water that was in the bound form.

The results with five tests at -7.5° C. showed the bound-water content to range from 34.4 to 38.4 percent, with a mean of 35.9 percent.

HEAT-OF-FUSION-OF-ICE METHOD

The heat-of-fusion-of-ice method was essentially that reported by Robinson (10). This method utilizes the fact that the melting of

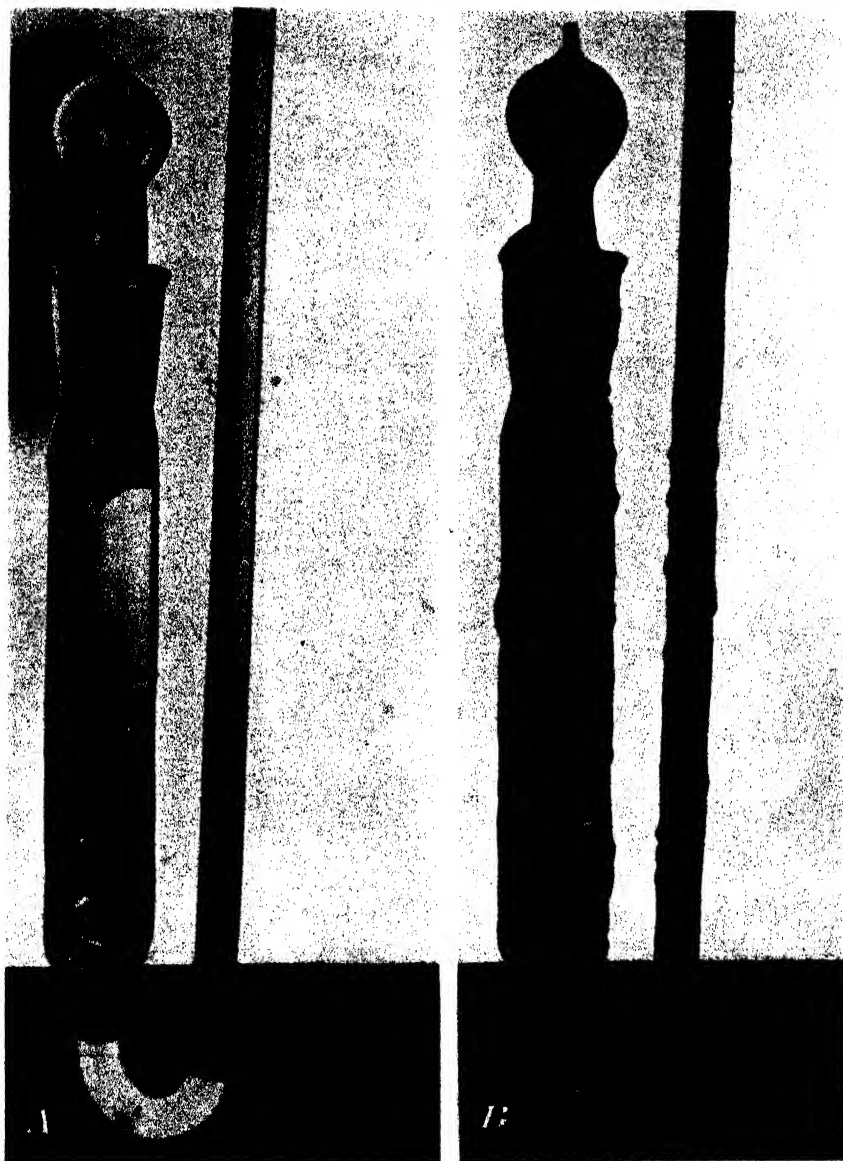


FIGURE 5.—Dilatometer bulb showing codling moth larvae (A) before freezing, and (B) after freezing. Note whitish color of larvae caused by freezing.

1 gm. of ice requires 80 calories of heat. By the use of a calorimeter (fig. 6) the heat given off by the thawing of frozen larvae is measured. This figure gives a basis for computing the frozen water that they contained. The total water content is then determined as was done in the dilatometric method, and the bound water computed by difference.

A 300-ml. silvered Dewar flask (*A*) was used as the calorimeter jacket. A small glass shell vial (*E*), held in place within the flask by means of a cork, served as a container for the calorimeter water. In all tests the water was carefully pipetted into the vial to avoid drops on the walls. A mechanical stirrer (*B*) was made from a glass rod by flattening it at one end and twisting it spirally to give adequate stirring without splashing. It was operated by a $\frac{2}{200}$ -horsepower motor.

The water sample used to determine the thermal capacity of the calorimeter and the samples of insects to determine the free-water content and specific heat were enclosed in tinfoil containers (fig. 7). A thermocouple was held in the middle of the insect samples.

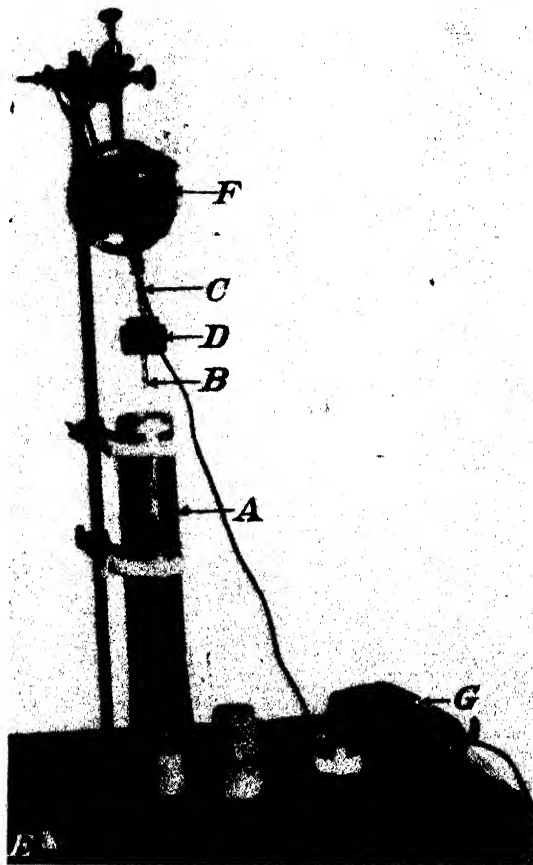


FIGURE 6.—Calorimeter, open: *A*, Dewar flask; *B*, glass stirrer; *C*, universal joint; *D*, cork stopper for Dewar flask and bearing for stirrer; *E*, container for calorimeter water; *F*, motor for stirrer; *G*, rheostat for motor.

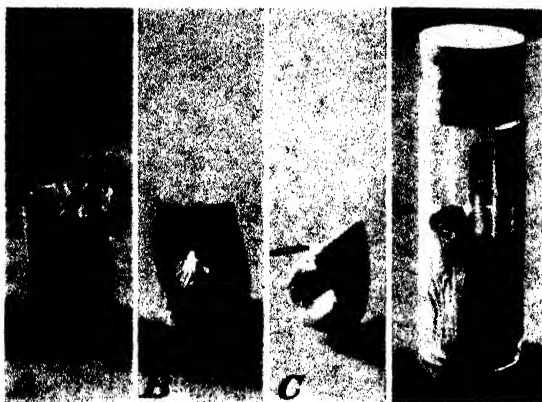


FIGURE 7.—Tinfoil container for specimens used in bound-water determinations by the heat-of-fusion method: *A*, tinfoil cylinder; *B*, cylinder after folding for freezing of specimens; *C*, cylinder as folded after specimen was frozen; *D*, glass vial container for specimens.

TABLE 5.—Bound-water content of codling moth larvae, determined by the heat-of-fusion-of-ice method

Temperature of frozen larvae, -30°C . Specific heat of larvae, 0.75 calorie per gram-degree centigrade

Weight of live larvae	Water content of larvae	Weight of tinfoil container	Temperature of calorimeter water		Bound water
			Initial	Final	
<i>Grams</i>	<i>Percent</i>	<i>Grams</i>	$^{\circ}\text{C}$.	$^{\circ}\text{C}$.	<i>Percent</i>
2.530	54.9	1.636	26.0	20.1	11.2
2.508	54.6	1.360	22.7	17.0	8.4
2.512	56.1	1.266	25.2	19.3	14.8
2.521	56.5	1.238	25.6	19.5	5.3
2.529	56.2	1.266	24.9	19.0	10.5
2.518	56.8	1.278	24.7	19.0	16.3
2.509	56.7	1.207	24.6	18.7	9.5
2.504	55.8	1.226	24.6	18.9	11.9
2.505	55.1	1.269	24.8	19.1	12.5
2.504	56.3	1.250	24.8	19.0	11.2
Mean 2.514	55.9	1.200	24.8	19.0	11.2

The results of 10 bound-water determinations by this method are given in table 5.

It will be noted that a lower value for bound water was obtained with the heat-of-fusion method at -30°C . than with the dilatometric method at -7.5° . A difference in results should exist, since the force of crystallization is more powerful in comparison with the force of water binding at -30° than at -7.5° .

SUMMARY

Studies have been made of the susceptibility of the hibernating larva of the codling moth (*Carpocapsa pomonella* (L.)) to low temperatures, as indicated by the temperature to which the insect can be undercooled before beginning to freeze. Studies have also been made, by two methods, of the bound-water content of the hibernating larva.

Unlike some insects, the codling moth cannot withstand more than one freezing.

The overwintering larva shows a pronounced tendency to undercool. The average undercooling temperatures for different groups of larvae ranged from -24.4°C . to -30.1° . The average rebound temperatures to which the temperatures of the insects rose after freezing started ranged from -5.0°C . to -11.2° .

No marked differences in susceptibility to low temperatures, as indicated by the undercooling temperatures, were shown by larvae from Washington, Colorado, Arkansas, Maryland, and Georgia.

The undercooling temperatures of the larvae were practically unaffected by the sex or weight of the larvae.

A study of the effect of exposing larvae to artificially created dry and humid conditions indicated no marked changes in their undercooling temperatures.

The undercooling temperatures of crossbred larvae were similar to those of their progenitors.

Undercooling is favored by the quiescent state of the larva during its hibernation period. The artificial movement of the larva greatly reduced undercooling.

The average bound-water content of codling moth larvae was 35.9 percent when determined by the dilatometric method at $-7.5^{\circ}\text{C}.$ and 11.2 percent by the heat-of-fusion-of-ice method at $-30^{\circ}\text{C}.$

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No. 11

THE CONSTRUCTION AND INSTALLATION OF THERMO- COUPLES FOR BIOLOGICAL RESEARCH¹

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INTRODUCTION

The thermocouple, when connected with a potentiometer, has several advantages over other instruments used to determine temperature changes in biological material. In the first place any temperature within its range may be determined instantly. Moreover, it is an improvement over the mercurial, spirit, or gas thermometer in that its small size permits measurement of temperatures in localized areas of living or dead tissue. Within limits, the distance from the thermocouple to the potentiometer is immaterial and a whole battery of thermocouples can be coupled with one potentiometer through a centrally located switchboard. Temperatures may, therefore, be recorded in rapid succession. Finally, thermocouples may be left in position indefinitely where corrosion does not take place.

In attempting to use this apparatus for biological research the writer discovered that many temperature readings were inaccurate. Later these inaccuracies were found to be due largely to incorrect mounting of the thermocouples. Further investigation revealed that many others who made temperature measurements in similar work with this equipment had used it without taking adequate precautions to prevent the same difficulty (1, 2, 3, 5).^{3 4}

Portable potentiometer indicators have found extensive use in industrial research and manufacturing. The measurement of the temperature of furnaces, of flowing liquids, and of rooms located at considerable distances from each other are among the multitude of ways in which they are employed. But under such conditions it is not difficult to set up thermocouples to measure temperatures correctly since the volume of material or the space held at a specific degree or

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³ Italic numbers in parentheses refer to Literature Cited, p. 341.

⁴ CALAHAN, C. L. THE PORTABLE POTENTIOMETER THERMOCOUPLE THERMO-ELECTRIC INDICATOR METHOD FOR RAPIDLY DETERMINING SMALL TEMPERATURE CHANGES IN LIMITED AREAS. N. H. Univ. Dept. Hort. 18 pp., illus. 1942. [Processed.]

range is comparatively large. In biological experiments it is frequently desirable to measure the temperature of limited areas, or differences in the temperature of tissues which are but a fraction of an inch apart.

Information dealing with the technique of using the portable potentiometer and installing thermocouples for this work is very scattered, incomplete, and contradictory. The material that follows was secured as a result of efforts to overcome difficulties arising in the construction of thermocouples and in mounting them to obtain accurate temperature readings in biological research experiments.

It is not intended to discuss in this paper the construction and operation of the potentiometer any more than is necessary. These problems have already been solved by the manufacturers of such instruments, and explained by authors of many textbooks of physics and chemistry (6, 7).⁵ It is necessary, however, to consider briefly the construction of the apparatus with which this work was done.

THE POTENTIOMETER

The most practical instrument found to measure temperatures of biological materials, so far, has proved to be a potentiometer calibrated directly in temperature units.

The potentiometer has been in general use since about 1912. In its simplest form it is well described by Duff (4). The one used by the writer, which is called a null balance instrument, measures thermocouple electromotive force by comparing it with the standardized voltage drop across a portion of a slide-wire, and uses a sensitive galvanometer to detect any unbalance. It consists essentially of a resistance wire of uniform and constant resistance throughout its length, in series with a battery and a current-adjusting rheostat. The voltage drop across the wire, or any part of it, is proportional to the current flow. It is necessary to use a current flow of constant value in this circuit as a standard of comparison. In order to check this constant flow it is necessary, at frequent intervals, to compare the voltage drop due to the battery current flowing across a calculated portion of slide-wire with the e. m. f. of a circuit carrying the opposing voltage of a standard cell (e. m. f. = 1.0190 volts). The galvanometer is used to detect any current which would flow if the potentials were not equal, and a rheostat in the battery circuit is adjusted until such equality is obtained. Any effects of variations of temperature on the e. m. f. of the battery circuit, with which the e. m. f. of the thermocouple is to be compared, can be eliminated completely before the latter is switched into the circuit in place of the standard cell.

Since no current flows from the thermocouple when the measuring instrument is balanced, the accuracy of measurement is usually independent of the length or diameter of the thermocouple leads, when they are properly installed. Therefore, when measuring temperatures of a group of thermocouples, it is not necessary to have all leads the same length, nor to "balance" the shorter leads with additional

⁵ LEEDS & NORTHRUP COMPANY. MICROMAX THERMOCOUPLE PYROMETERS FOR MEASUREMENT AND CONTROL. Leeds & Northrup Co. Cat. N-33A, 57 pp., illus. Philadelphia. 1942.

resistance. Certain limitations concerning length and size of leads will be discussed later.

Calibration of this potentiometer depends upon stable and fixed resistance coils and slide-wires rather than on delicate deflection meters. The sensitive galvanometer is used as a null balance detector only and is not calibrated.⁶

CONSTRUCTION OF THERMOCOUPLES

The terms "thermocouple," "couple," and "junction" are used interchangeably in this paper. A thermocouple, as ordinarily described in physics, consists of two wires of unlike material fused or soldered together to make two junctions. The copper wire is then cut in two at the center to serve as leads to the measuring instrument. If the two junctions are at different temperatures, there is a voltage between the two lead-wires and this is measured. The measurements are standardized by placing one of the junctions in melting ice at a constant temperature.

The type of thermocouple, or, strictly speaking, the portion of thermocouple employed in the following investigation is made up of two wires each of a different metal, welded, fused, or soldered together at one end. An electromotive force, or electrical pressure, is developed at the point of junction of the wires and is proportional to the temperature difference between this point, the so-called "hot" junction, and the reference or "cold" junctions, located in this case in the instrument to which the free ends of the wires (lead-wires) extend. At this point it should be understood that it is possible, and necessary, to solder the hot junction and short leads of No. 30 or finer wire, to larger lead-wires of the same metals, and extend these to the instrument if the latter is more than 15 feet away. The potentiometer employed is also equipped with an automatic reference junction compensator which eliminates the need for the ice bath described above, a decided convenience for tests run in the field and over a long period of time.

To be most useful in obtaining internal plant temperature, thermocouples must of necessity be as small in diameter as it is practical to make them so that there will be the least possible injury to the tissue in which they are inserted. Number 30 copper-constantan enameled wire was selected as about the smallest that could be used conveniently in this work. Number 38 and possibly finer can be obtained,⁷ but these would require such sensitive galvanometers that they could be used only under carefully controlled laboratory conditions and are too easily broken to be useful for temperature studies under field conditions extending over a long period.

Each potentiometer is calibrated for thermocouples made of specific materials.

Several batteries of thermocouples were made for these experiments.

⁶ The instrument referred to was a manually operated Leeds & Northrup portable potentiometer indicator with automatic reference junction compensator. It is calibrated -40° to 120° F., for use with copper-constantan thermocouples (1938 calibration).

⁷ BAKER & CO., INC. FINE WIRES. 15 pp. New York, San Francisco, and Chicago. [No date.]

The copper and the constantan lead-wires from each thermocouple in a battery were the same length, but those in different batteries ranged in length from 3 feet up to 115 feet. Short wires were cut in pairs and each pair was wrapped on a spool. Couples with long lead-wires were marked by attaching identically numbered tags at both ends, but a whole battery of these thermocouples and leads was wrapped on one spool. When unwound it was, in effect, a cable. This practice removed the necessity of keeping the small, easily tangled lead-wires separated from each other throughout their whole length, and greatly simplified installation.

In constructing a thermocouple the enamel was first scraped off one end of each of two wires. Fine emery cloth is an excellent material with which to remove enamel. These cleaned ends were twisted tightly together for several turns, and were then fused by holding them in the flame of a medium hot gas burner with the twisted ends turned upward at an angle of from 60° to 70° above the horizontal position.

Copper has a melting point of $1,083^{\circ}$ C. and will, therefore, melt before the constantan, an alloy of nickel and copper, which has a melting point of $1,290^{\circ}$ C. At its melting point the copper fuses around the constantan. This point can best be observed by watching for it through a large lens. A reading glass is satisfactory. Wires must be removed from the flame the instant fusion takes place, and before a droplet of copper forms, or before the wire melts apart. Small droplets do not appear to interfere with the accuracy of readings, but do interfere with the insertion of the thermocouple into plant material, and could, if they were large enough, result in excessive thermal conduction because of their large heat capacity. Any piece of constantan which protrudes beyond the tip of the fused junction can be cut off easily with a sharp knife if the projection is first laid on a solid surface.

Satisfactory thermocouples can also be made by cleaning the ends of the wires, twisting them together for a short distance, and then soldering the twisted portion to prevent corrosion and hold it firm. Thermocouples used in these experiments were either fused, or fused and soldered, and they gave equally good results. A concentrated zinc chloride flux, used in the process of soldering, was very effective. The fused junctions or twisted wires were first dipped into the zinc chloride flux, then inserted into solder heated to a temperature considerably above the melting point, and immediately withdrawn. An excellent junction is produced by this method, but any zinc chloride remaining on it should be rinsed off in water to prevent corrosion. A more durable and equally efficient couple can be made by fusing the ends of the wires and soldering them together up to one-eighth of an inch from the junction. However, any additional material placed around or near the junction requires extra space, makes installation difficult, and introduces additional error due to thermal conduction.

If a sufficient length of lead-wires, including the junction, can be embedded at the same depth in the object to be measured, it will not be necessary to fuse the extreme tips of wires for junctions to be used in measuring the temperature of objects, as will be shown later, since this practice prevents thermal conduction toward or away from the junction. In measuring the temperatures of thin or small

objects it would, of course, be preferable to make the junction occupy as little space as possible, which can be done by fusing the ends of the wires by means of an electric arc.

INSTALLATION OF THERMOCOUPLES

WORK OF OTHER INVESTIGATORS

Thermocouples have frequently been used to measure the internal temperature of plants. Clum (1) describes apparatus for and methods of measuring leaf temperatures. He used No. 36 copper-constantan wire in his experiments. The thermocouples were fused with an electric current and were insulated with "Radiolac." Lead-wires at an undesignated distance from the junctions were also protected by running them through cotton sleeving. Junctions were inserted into the mesophyll of the leaf through a small hole in the lower epidermis and held in place with a small wire clamp slipped over the edge of the leaf and over the thermocouple wires a short distance from the junction. By this method only a few millimeters of junction and adjoining lead-wires would be embedded in plant tissue.

Wallace and Clum (11), in later work on leaf temperatures, used thermopiles consisting of clusters of five thermocouples of No. 38 copper-constantan wire. These were mounted on a wire clip within a wire frame 6 mm. square which was pressed against the lower surface of the leaf when the clip was closed on the leaf. This would seem to be an excellent method of obtaining the temperature at the lower surface of leaves, but would not necessarily determine the internal temperature of a leaf at any given instant, since thermal conduction could affect the temperature of the couple on the leaf but not that of the couple suspended in air. However, it is obvious that the No. 38 wire used would introduce far less error than No. 30 wire.

Eaton and Belden (5) also used No. 36 copper-constantan wire in their measurements. Their thermocouple wires were enclosed in "semiflexible tubing" from which the junctions protruded about 1 inch. The upper surface of a leaf was folded against the junction with a pair of cork-tipped tongs and the reading on the galvanometer taken immediately. The results were in terms of "the departures of the leaf temperatures from the temperature of the air," which were obtained from a shaded thermocouple (5, p. 35).

Curtis (2, 3) used No. 30 copper-constantan thermocouples which he "threaded" through the leaves. All except about 1 cm. of junction and lead-wires was insulated. In this work the junctions of the thermocouples and short lengths of adjacent lead-wires were in contact with the lower surface of the leaf. In wire of this size the thermal conduction factor exerts a great influence on temperature readings, as will be shown. Insulating lead-wires to within 1 cm. of the junction, on the basis of the following work, is of questionable value, especially in recording temperatures that can change as rapidly as they do in leaves. Insulating material would absorb heat during a rise in temperature at the junction, and would permit it to be conducted back to the junction as the latter point becomes cooler. This would definitely tend to straighten the temperature curve.

Patton and Feagan (8) devised an ingenious method of measuring

the fall in temperature of a liquid flowing through a tube. Thermocouple junctions were soldered in the ends of slots machined in the wall of a metal tube parallel to its length. These junctions were located very near the inner wall of the tube. Lead-wires were then run through a smaller tube laid in the machined slot. This smaller tube was also soldered in place and the solder ground down at the surface to restore the original outer curvature of the pipe. By this method the thermocouple junction and about 3 inches of lead-wires were exposed to the same temperature, and false readings due to thermal conductivity away from the junctions were eliminated.

OBJECTIVES

Because of their sensitivity, thermocouples have long been of outstanding value in measuring plant temperatures. The writer wished to use them both in trunks and branches of trees and at various distances above and below ground. However, suspicion arose as to possible variability in readings due to thermal conduction along the lead-wires. Many readings were taken in comparison with those of a mercury medical thermometer placed under the human tongue. The readings averaged from 1° to $1\frac{1}{2}^{\circ}$ lower for the thermocouples made of No. 30 copper-constantan wire, but when the lead-wires adjacent to the junction were bent into the form of a compact S the error was eliminated.

A series of experiments was carried out to determine whether there was thermal conduction of heat away from the thermocouple junction along the lead-wires to a colder point some distance away, or vice versa, and whether such conduction would affect temperature readings when copper-constantan thermocouples of No. 30 gage were used. Studies also were made to find a method of inserting thermocouples in plant tissue in such a way as to avoid incorrect readings, or at least to reduce the inaccuracies to a minimum.

METHOD OF PROCEDURE

Equipment was set up to determine what effect would be produced by conduction of heat toward or away from the thermocouple junction. For this work several thermocouples of No. 30 copper and constantan wire were prepared by welding in a gas flame. Some of these were dipped in solder and others left as welded to test the effect of soldering. All gave identical temperature readings when checked against a standard couple. The comparison was made by suspending the couples to a depth of 6 inches in water that had stood for 48 hours in a room at nearly constant temperature. A standardized thermometer was used as a further check.

Thermocouples with lead-wires 115 feet long gave as accurate readings on the potentiometer previously described as thermocouples with 4-foot lead-wires at temperatures of 0° F. or above. However, the galvanometer needle was less sensitive to thermocouples with the long leads during the measurement of subzero temperatures. This was especially important since the nearest peach trees in which couples were to be inserted were located 100 feet from a room in which the instrument was to be placed in order to operate it in cold weather. Larger lead-wires of copper and constantan were not available. But

when low temperatures are to be measured at a distance of more than 15 feet from the instrument it would be preferable to make thermocouples and short lead-wires of No. 30 copper-constantan wires and solder them to No. 16 gage insulated leads of the same metals to eliminate high resistance in the galvanometer circuit. One group of tests for thermal conductivity was carried out with 4-foot couples, the other with 9-foot couples. The results are recorded in the data that follows.

TESTS SHOWING CONDUCTION ALONG LEAD-WIRES IN AIR TO A JUNCTION
SUSPENDED IN WATER

In the first set of experiments a thermocouple was suspended one-half inch deep in a gallon jar of water that had stood in a room of nearly constant temperature for 48 hours (fig. 1). The temperature of the water both before and after the experiment was 77.75° F. By means of a measuring stick mounted besides the apparatus it was possible to indicate the exact point above the water at which the lead-wires were grasped by the fingers of the operator. This method was sufficiently accurate to show the desired results since the chief concern was whether or not thermal conduction influenced temperature readings during these first experiments rather than the exact amount of variation. Obviously there is opportunity for variation in the temperature of the fingers, particularly where short lengths of wire are grasped, but this would minimize rather than amplify the results

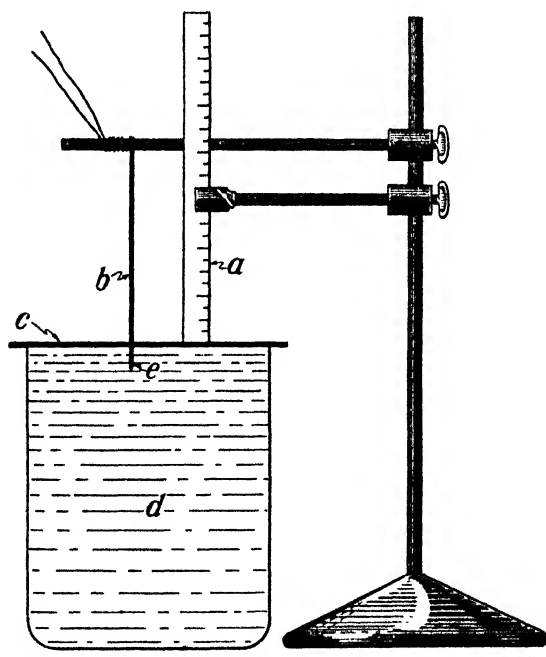


FIGURE 1.—Apparatus used to test for thermal conductivity along lead-wires to a thermocouple junction suspended in water: (a) Ruler; b, thermocouple lead-wires that were grasped at various distances above c; c, insulating paper; d, water; e, thermocouple junction.

under the conditions of the experiment that follows. The tabulation given below shows the results of the experiment to determine the conductivity of heat from fingers, through metal in air and water, to a thermocouple junction.

*Distance above water at which wires were grasped
by fingers*

Temperature reading after 20 seconds

<i>Inches</i>	<i>° F.</i>
3	77.75
2	77.75
1	77.75
$\frac{1}{2}$	78.50
$\frac{1}{4}$	81.25

This tabulation indicates that heat from the fingers was conducted along the No. 30 wires and was not dissipated into the air or water and thus prevented from reaching junctions located less than 1 inch from its source. The thermal differential or the difference between the temperature of the fingers (96.5° F.) and of the water (77.75°) was only 18.75° F. at the beginning of this experiment.

EVIDENCE OF THERMAL CONDUCTION IN EXPERIMENTS WITH LIVE WOOD OF SUGAR MAPLE

Trials were carried out with a slab one-quarter inch thick cut from a young, dormant sugar maple tree on a winter day when the temperature was well above freezing. The wood was very moist. Sap flowed freely from the stump after the tree was cut. A hole 1 mm. in diameter was drilled through the slab and the junction of the thermocouple was inserted. The junction was then pushed on through a double thickness of heavy paper insulation beyond which it was grasped by the operator whose fingers registered 96.50° F. (See fig. 2). The recorded temperature inside the slab was 66° F. at the beginning and 70° after completion of the tests.

The effect due to conduction of the quarter inch of cold wood on the temperature of the thermocouple point when the point was moved to various distances from the wood is shown in the tabulation given below. The temperature of the fingers on the thermocouple outside the wood was 96.5° at the beginning and at the end of the test; the temperature of the inside of the slab was 66° at the beginning and 70° at the end of the test; the temperature of the air in the room was 79°.

Distance thermocouple junction protruded beyond cold slab

Temperature of fingers on thermocouple points at different distances from slab

<i>Inches</i>	<i>° F.</i>
$\frac{1}{4}$	85.5
$\frac{1}{2}$	92
$\frac{3}{4}$	92.25
1	94.75
$1\frac{1}{4}$	95
$1\frac{1}{2}$	96
2	96.5
$2\frac{1}{2}$	96.5

With a difference of 30.5° F. between the temperature in the slab and that of the fingers it is apparent from these figures that at a distance of $\frac{1}{4}$ inch the temperature reading of the object at the ther-

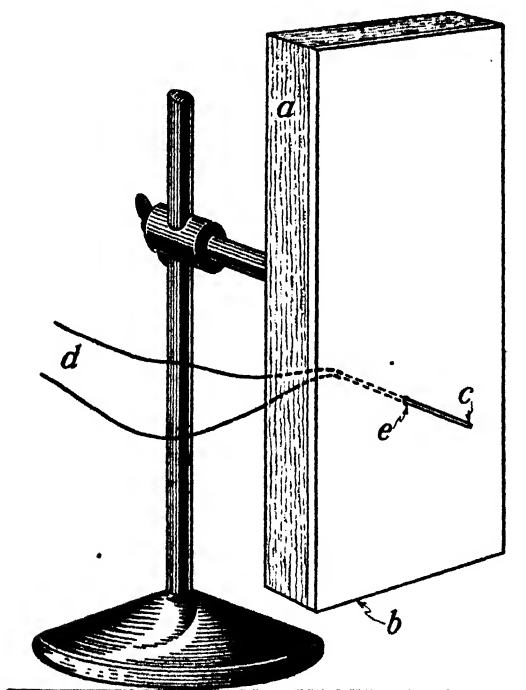


FIGURE 2.—Apparatus used to test the effect on the temperature at a thermocouple when lead-wires from the junction were passed through green maple wood which was colder than the temperature recorded at the junction: *a*, Maple slab; *b*, insulating paper; *c*, thermocouple junction; *d*, lead-wires; *e*, hole through which lead-wires were pulled to various distances beyond slab. The thermocouple was grasped by the fingers when it was one-fourth inch from insulation paper (*b*) placed over the slab (*a*). The distance from the junction to the insulation paper was increased 2 inches before the true temperature of the fingertips could be recorded.

mocouple junction is thrown off 11° , and at $1\frac{1}{2}$ inches it is off 0.5° . The temperature of the fingers was used in order to measure results with a greater temperature differential than was possible in the air of the room.

An experiment was made to determine the effect of high temperature at the surface of a limb on thermocouple points inserted to different depths in holes drilled into the limb.

For this work a limb of live sugar maple 4 inches in diameter and 3 feet long was selected. Three holes, each 1 mm. in diameter, were drilled into it at right angles to its length. These holes were $\frac{1}{16}$, $\frac{1}{8}$, and $\frac{1}{4}$ inch deep, respectively. Thermocouples were inserted in them and a small amount of cotton forced in at the opening in the bark to hold the couples firmly in place. Readings were taken immediately after each couple was inserted. The operator then grasped the wires at the point where they entered the limb and readings were taken again. All temperatures were recorded within an interval of 3 minutes, beginning with the thermocouple which was $\frac{1}{16}$ of an inch deep. The temperature of the room in which the limb stood was 79° F. at

the beginning of the test and the temperature of the fingers, 96.5°. The results of the experiment are shown in the accompanying tabulation.

Depth of hole in which thermocouple point was inserted	Temperature of limb as it stood in room	Temperature recorded by thermocouple 3 minutes after start of test when lead-wires were grasped by fingers at point of emergence
Inches	° F.	° F.
$\frac{3}{16}$	47.5	65
$\frac{3}{8}$	41.5	50.75
$\frac{1}{2}$	36.0	39.5

EFFECT OF INSERTING THERMOCOUPLES IN HOLES OF DIFFERENT SIZES

A test was made to determine the effect on temperature recordings of inserting thermocouples in different-sized holes in a tree and plugging the openings in different ways where the lead-wires emerged. Holes 1.5, 1.7, and 4 mm. in diameter were drilled at the same level on the north side of a shaded peach tree. A shaded tree was selected so that sunlight would not influence the temperature of the couples. All holes were drilled to a depth of 1.5 inches. Table 1 gives the results of this test.

The differences obtained with the different sized holes and plugs, as shown by table 1, are not particularly significant because of the small variation in temperature of the air and in the wood, but they might indicate that the true temperature of the tree at $1\frac{1}{2}$ inches deep was not being measured accurately and that the junctions as they were inserted were affected by the temperature of the air. Differences would probably have been greater at higher temperatures or during more rapid changes in temperature. Discrepancies are probably due in part to the curvature of the tree and differences in the structure of the wood.

TABLE 1.—Temperatures recorded when thermocouples were inserted in holes of different size in peach tree and held in place by different methods

Date and time of day	Temperature in holes of different sizes with couples held in place as indicated				
	1.5 mm.; cotton plug	1.7 mm.; cotton plug	4 mm.; cot- ton plug	1.7 mm.; cotton plug grafting wax	Tempera- ture of air
Dec. 18:	°F.	°F.	°F.	°F.	°F.
6:00 p. m.	13.75	13.75	14.0	13.75	12.0
7:15 p. m.	10.5	10.5	10.5	10.0	8.75
9:00 p. m.	7.25	8.0	8.0	7.5	5.5
Dec. 19:					
7:00 a. m.	-3.5	-3.0	-3.0	-3.0	-2.5
12:00 m.	4.0	4.0	4.0	4.0	5.0
1:45 p. m.	5.0	5.0	5.0	5.0	4.5
6:00 p. m.	-2.0	-1.5	-1.5	-1.5	-4.0
11:00 p. m.	-11.0	-11.0	-11.5	-11.5	-12.0
Dec. 20:					
8:30 a. m.	-15.5	-15.5	-15.25	-15.5	-14.25
10:15 a. m.	-10.0	-10.25	-10.0	-10.5	-7.0
12:45 p. m.	-1.0	-1.25	-1.5	-1.5	4.25
5:30 p. m.	4.25	4.25	4.25	4.25	3.75

A COMPARISON OF METHODS OF INSERTING THERMOCOUPLES

As a result of the experiments just described the writer concluded that it is necessary to place at least 3 inches of a No. 30 copper-constantan thermocouple wire and junction in the same level of plant tissue, or at a place where it will be subjected to the same temperature, in order to obtain accurate readings. This distance was selected to provide a margin of safety for extreme changes in temperature in tissue of different moisture content.

To test the validity of this conclusion a piece of live apple limb 16 inches long and 5 inches in diameter was selected and cut off. Two sets of four holes, each 2.5 mm. in diameter, were drilled in it. The first set of four holes were drilled in one end to a depth of 3 inches. These were $\frac{1}{4}$, $\frac{1}{2}$, 1 and 2 inches from the side of the limb and parallel to it, but so distributed that one hole was not under another. The second set of four holes of equal diameter were drilled $\frac{1}{4}$, $\frac{1}{2}$, 1, and 2 inches deep into the limb at right angles to its length and surface. These holes were also distributed part way around the limb. Thermocouples were inserted in the holes. A plug of cotton and a light covering of grafting wax were placed around each pair of wires where it emerged from the limb. The limb with the thermocouples inserted was placed in a thermostatically controlled refrigerator capable of being cooled to -40° F. Another thermocouple to indicate air temperature was placed beside it. The lead-wires were passed through a hole in the wall of the refrigerator to the potentiometer located outside. Temperatures were recorded every 15 minutes from the time the compressor was started until it was shut off and the inside of the chamber had again reached the temperature of the room. During the time the temperature was rising, the door fasteners of the refrigerator were released slightly to allow warm air to enter and cold air to drain out; however, the air inside was circulated sufficiently by a large fan so that it was of uniform temperature at any given moment. After the couples had been removed the limb was cut to check the position of points at the base of the 3-inch holes. The results with thermocouples set at $\frac{1}{4}$ inch in each of the two tests are illustrated in fig. 3.

The results of this experiment indicate that during rapid changes in temperature at higher levels, thermocouples set at shallow depths, with but $\frac{1}{4}$ inch of wire embedded in the tree, may read from 3° to 9° lower or higher than temperatures registered on a couple 3 inches of which is embedded at the same depth. When temperatures are changing at a lower rate the difference between readings of thermocouples in the tree and those in air become more nearly the same regardless of how the instruments are inserted in the tree, since there is less conduction of heat toward or away from these junctions. The conditions indicated in figure 3 for the period between 5:30 and 8:00 p. m. are comparable to those sometimes occurring in an orchard between 9:00 a. m. and 1:00 p. m. on a bright, sunshiny day in mid-winter. Any thermocouple set in a hole $\frac{1}{4}$ inch deep drilled at right angles to the bark, as indicated in figure 3, would not register correctly because of the great amount of heat conducted along the wire to the junction. When 3 inches of the wires are embedded at $\frac{1}{4}$ inch depth, hot or cold air striking them where they enter the tree would

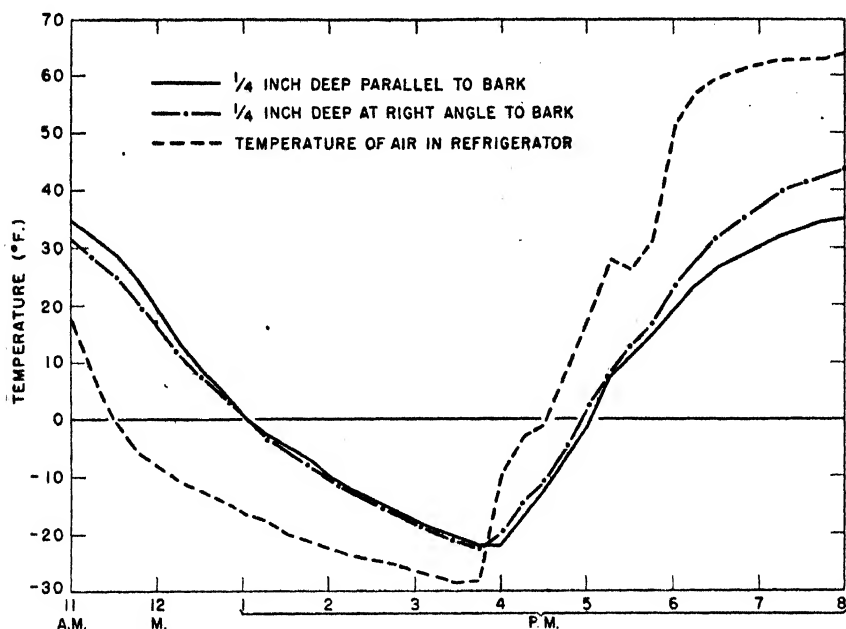


FIGURE 3.—Effect of thermal conduction on the temperature at a thermocouple junction installed $\frac{1}{4}$ inch deep at a right angle to the surface of an apple limb 5 inches in diameter as compared with its effect at another junction, also set $\frac{1}{4}$ inch deep, but inserted parallel to the surface of the limb for a distance of 3 inches.

be dissipated before reaching the junction, resulting in a more nearly perfect record of conditions at that location. The accuracy of this statement is indicated by figure 4, which shows the results obtained by setting the couples about 2 inches deep. When the limb was cut, the point of couple No. 1 proved to be $1\frac{3}{4}$ inches deep, which accounts for the slight discrepancy in the readings. However, at this depth the readings of both thermocouples follow the same curve with but little variation, indicating that little influence, if any, resulted from conduction of heat along the wires toward, or away from, these points.

Since it would be extremely difficult to insert a thermocouple and 3 inches of adjoining lead-wires parallel to the length of a tree trunk at a depth much greater than the cambium, the following method was devised for measuring temperatures deeper in the trunk. One-fourth-inch bamboo reed was cut into lengths about 2 inches longer than the radius of the tree to be measured, and a small steel wire was pushed through them to open the nodes. A hole was drilled in the trunk into which the reed was inserted for a trial fitting. The depth to which the reed extended was carefully measured, after which it was withdrawn and shallow, close spiral notches were cut in its outside wall at distances which represented the depth at which thermocouples were to be placed. The thermocouples and lead-wires were then inserted in the reed and were allowed to protrude from it at the desired point for a distance of 3 inches. Between three and four wraps around the $\frac{1}{4}$ -inch reed will, even when slightly separated, occupy little

space, but will absorb the 3 inches of lead-wires and will therefore reduce to a minimum the amount of thermal conduction along the lead-wires toward or away from the junction. The whole unit was then dipped in shellac, and when it dried the wires were held firmly in place for inserting in the tree. If the unit has more than one thermocouple on it, lead-wires should be carefully labeled. A small amount of grafting wax was applied where lead-wires emerged from the tree.

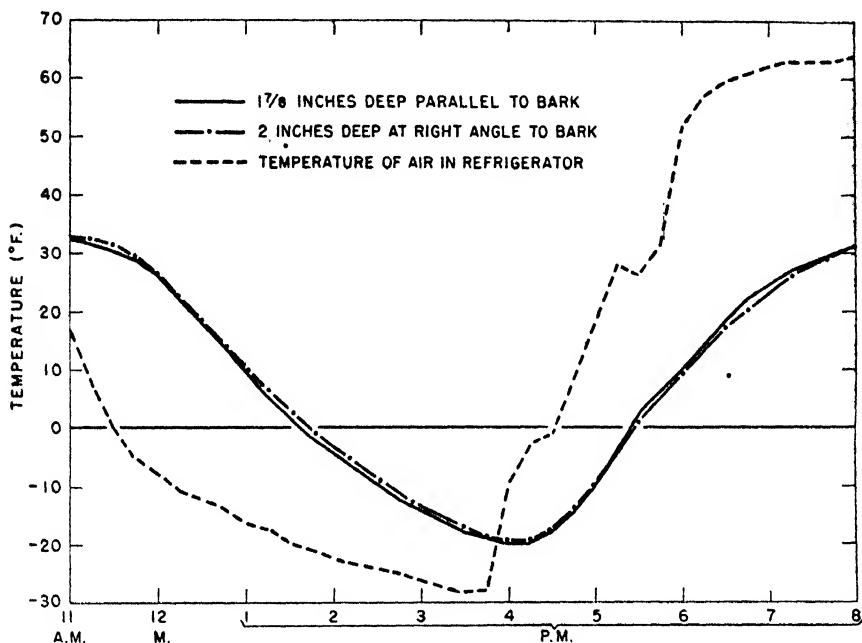


FIGURE 4.—Effect of thermal conduction on the temperature of a thermocouple when junctions were installed as indicated in figure 3 but at a depth of about 2 inches. The temperatures recorded in figures 3 and 4 were taken on the same apple limb at the same time.

In the event bamboo reeds are not available, small, straight branches can be cut from young, live wood not over $\frac{1}{4}$ -inch in diameter. When the bark is peeled off these they can be split lengthwise and the pith removed, after which they can be used like the bamboo reeds described above.

An excellent time of year to place thermocouples in the cambium layers of trees to study winter temperatures is when the bark slips in August. A hard steel wire sharpened on one end and pushed vertically along the trunk into the cambium can be forced along the latter very easily at that time of year. If inserted immediately after the wire is withdrawn, a thermocouple can, without difficulty, be pushed into position. A small amount of grafting wax can be brushed on the hole where the wires emerge, and they will soon become firmly established.

SUMMARY AND CONCLUSIONS

The potentiometer-thermocouple method of measuring the internal temperature of plants is accurate if the thermocouples are properly installed. Within working limitations the size of the hole in which a couple is placed is not as important as other factors of installation, but from the standpoint of injury to plants the smallest hole in which a junction can be inserted is best.

In order to obtain an accurate reading of temperature in live wood tissue during rapidly rising or falling temperature it is necessary that about 3 inches of No. 30 copper-constantan lead-wire be embedded at the same depth as the thermocouple junction, in order to "isolate" the junction and dissipate the effect of heat which tends to be conducted toward or away from that point. Plant tissues vary greatly in texture and structure as well as in moisture content at different periods of the year. Since measurements taken in these studies were made on green maple filled with sap, and on comparatively dry apple wood, it is suggested that this 3-inch distance would probably be satisfactory in any plant tissue when wire of the same description is used.

A method is described for inserting thermocouples in tree trunks. It is desirable that the plug which holds the junctions firmly in place in the plant should be waterproof substance. Grafting wax is suggested.

Thermocouples should be installed in the cambium of trees in a position parallel with the length of trunk or limb to insure more accurate readings of temperatures on any one side of the tree.

Thermocouples should be constructed of as fine wire as it is possible to use in order to avoid thermal conductivity toward or away from the junctions under conditions of changing temperature.

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BLOOD LEVELS OF CALCIUM AND INORGANIC PHOSPHORUS IN HEREFORD CATTLE¹

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INTRODUCTION

A possible relation between fertility and the levels of calcium and phosphorus in the blood of cattle has been suggested by various investigators. Proof of such a relation is, however, dependent upon a definite knowledge of the normal levels of these minerals. Such levels have been found for some breeds of cattle, but there is no general agreement in the results reported. The study here presented was undertaken to determine such standards for Hereford range cattle as a preliminary to the larger study of the relation of calcium and phosphorus blood levels to fertility.

LITERATURE REVIEW

The calcium and phosphorus blood levels of cattle recorded in the literature clearly indicated that type of feed, age, and breed influenced the blood level of those minerals. No reference was found, however, to the influence of sex on these levels. For the most part published data were based on small groups.

Haag and Jones (6)² reported the phosphorus level of blood plasma for mature cattle to be 5.2 mg. per 100 ml. This level does not agree with one reported by Johnson (7), which is 4.33 mg. per 100 ml. of whole blood for dairy heifers and cows, a mixed age group. Johnson found no difference for milking cows and dry cows, or for different breeds.

Robinson and Huffman (11) obtained 5.87 mg. of phosphorus per 100 ml. of blood plasma, for a herd of beef cattle not homogeneous as to age, the youngest being about a year old. Palmer, Cunningham, and Eckles (10) found the average inorganic blood phosphorus of a small group of calves to be 7.26 mg. per 100 ml. of plasma.

Beeson et al. (2), working with growing and fattening beef calves, found 6 mg. of blood phosphorus per 100 ml. of plasma adequate. They studied a homogeneous age group, but the results cannot be compared with those for a beef-range group because of the different type of feed.

Malan et al. (9), in the Union of South Africa, found that beef heifers approaching 2 years of age showed a normal phosphorus level of 5 mg. per 100 ml. of whole blood. This was a homogeneous age group, on pasture supplemented with phosphorus.

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² Italic numbers in parentheses refer to Literature Cited, p. 362.

Black et al. (3), working with a mixed age group, reported the point at which phosphorus deficiency existed in range cattle, used in the experiment at the King ranch in southern Texas, to be 4 mg. per 100 ml. of blood.

Knox et al. (8) found the phosphorus in wet cows of Hereford range cattle to be below 4 mg. per 100 ml. of plasma, except in a small group of dry cows and steers, in which the level closely approached 4 mg. per 100 ml. of plasma.

Haag and Jones (6) reported the calcium blood level for normal mature dairy cattle to be 9.99 mg. per 100 ml. of plasma. Allcroft and Green (7) gave the analyses of 139 normal dairy cows and reported the range for the blood-calcium level to be 8.65 to 11.65 mg. per 100 ml. of serum. However, no levels were given for range beef cattle.

A corresponding lack of agreement is evident for other phases of the investigation of blood phosphorus and calcium. Shohl (14 p. 185) found that when inorganic blood phosphorus was high blood calcium was low, and vice versa. VanLandingham et al. (13), on the other hand, reported that age and level of phosphorus intake were without effect upon the calcium content of the whole blood of growing dairy heifers. Greaves et al. (5) also stated that phosphorus intake had little effect upon the calcium content of yearling beef steers, but that there was a close correlation between phosphorus intake and the inorganic phosphorus of blood. Black et al. (3) reported a similar correlation between phosphorus intake and the inorganic phosphorus intake and the inorganic phosphorus of blood.

MATERIALS AND METHODS

A large herd of Hereford cattle running on a 70,000-acre pasture was available for this study. The cows bled in the experiment were at the end of lactation and the beginning of gestation. The cattle subsisted for 7 to 12 months of the year on the available pasture plants with supplemental feed during the winter months.

This Wyoming pasture lies east of the mountains at an altitude of 6,000 to 7,000 feet above sea level. The forage is composed for the most part of mixed grasses, chiefly grama, needle, and buffalo. The comparatively uniform altitude, topography, and flora of this area are considered of importance, since it is recognized that the phosphorus content of forage varies with the composition of the soil upon which it is produced and with the growing season.

The calcium and phosphorus content of the range forage was not determined; unless the person doing the sampling was very familiar with the range and grazing habits of the cattle, it would be difficult on so large a range to sample accurately the forage consumed. There was no clinical evidence in the herd to show that the intake of these minerals was inadequate.

For the purpose of this study the cattle were divided into four groups, based on sex and age: (1) yearling bulls; (2) herd bulls—those older than group 1; (3) 2-year old heifers; and (4) aged cows—those older than group 3. In stage of development the yearling bulls are comparable to the 2-year-old heifers.

Blood samples for analyses for calcium and phosphorus content were taken in connection with the routine care of the herd. Whenever cattle were being run through the chutes for such operations as change

of pasture, branding, spraying, or dipping, it was planned to take samples of jugular blood from every tenth animal going through the chutes. Owing, however, to the exigencies of such operations it was sometimes the fifth or even the eleventh that was sampled. The distribution may be considered as adequate for securing representative samples of blood.

The general procedure was to collect jugular blood in glass vials and use the serum for the calcium and inorganic phosphorus determinations. The analyses were begun within 8 hours of collection of the blood, and even when a large number of samples were being handled the analyses were completed within 24 hours.

Inorganic phosphorus determinations were made by the method of Fiske and Subbarow (4); calcium determinations were made by the method of Roe and Kahn (12); both were adapted to the Klett photoelectric colorimeter.

Precautions were taken to check the reliability of determinations by including, in a series of determinations, a check sample of known phosphorus and calcium content; this was done each time new reagents were made up. The check samples were prepared in approximately 10-percent trichloroacetic acid, in such a manner that 5-ml. aliquots were equivalent to blood filtrates containing 3.5 to 8 mg. of inorganic phosphorus per 100 ml. and 9 to 11 mg. of calcium per 100 ml.

Calcium and phosphorus were measured in terms of milligrams per 100 ml. of blood serum, for brevity expressed as milligram percent (mg. percent.)

A total of 560 determinations was made. Statistical analysis was used on the entire series, but only tables of statistics pertinent to this discussion are presented.

EXPERIMENTAL DATA

The normal blood inorganic phosphorus and calcium levels of cattle reported in the literature show little agreement largely because of the influence of age, feed, breed, and the small experimental groups of cattle used. In the work that follows, it was attempted to take into consideration these factors, and in addition to note the influence of sex on the normal blood-phosphorus and calcium levels in Hereford cattle raised in the same geographical location and of similar breeding.

Statistical studies of the blood-phosphorus analyses are presented in table 1 for the four general classes of animals under study. The groups were large enough to warrant considerable reliance upon the trends and differences which are indicated.

TABLE 1.—*Blood-phosphorus level of cattle Hereford expressed as milligram percent.*¹

Class	\bar{x}	σ	$\sigma\bar{x}$	n
Yearling bulls	7.30	1.46	0.103	203
Herd bulls	4.76	.81	.099	67
2-year-old heifers	5.07	.78	.063	155
Aged cows	4.89	1.17	.101	135

¹ \bar{x} = Arithmetic mean average; σ = the standard error of an individual observation; $\sigma\bar{x}$ = the standard error of a corresponding mean; n = the number of animals comprising the sample (number of determinations).

The differences between means of blood-phosphorus levels (table 1) of the four homogeneous classes are indicated in table 2.

TABLE 2.—Comparison of blood-phosphorus levels in different classes of animals.

Classes	Difference between means	σd^1
Yearling bulls vs. herd bulls.....	² 2.54	0.14
Yearling bulls vs. 2-year-old heifers.....	² 2.23	.14
Yearling bulls vs. aged cows.....	² 2.41	.12
Herd bulls vs. 2-year-old heifers.....	² .31	.14
Herd bulls vs. aged cows.....	.13	.11
2-year-old heifers vs. aged cows.....	.18	.12

¹ σd = the standard error of an indicated difference.

² = Significance at the 1-percent level.

³ = Significance at the 5-percent level.

The data presented in tables 1 and 2 show that the amount of phosphorus in the blood of yearling bulls was high as compared to that of other classes; that the phosphorus content of the blood of the males was greater than that of the females; and that the quantity of phosphorus diminishes with age in Hereford cattle. This relationship with age is stronger in the male animal.

Results of the blood-calcium analyses are presented in table 3 for the same four classes, and the differences between the means of the blood-calcium levels are given in table 4. The sample sizes for the calcium determinations were smaller than those employed in the phosphorus determinations, because a calcium deficiency seldom occurs in roughage-consuming animals, and the numbers were large enough to assign mean levels.

TABLE 3.—Blood-calcium level of cattle Hereford expressed as milligram percent ¹

Class	\bar{x}	σ	$\sigma\bar{x}$	n
Yearling bulls.....	10.46	1.63	0.238	48
Herd bulls.....	13.03	1.33	.250	27
2-year-old heifers.....	9.13	1.39	.158	78
Aged cows.....	9.62	1.81	.249	54

¹ \bar{x} = Arithmetic mean average; σ = the standard error of an individual observation; $\sigma\bar{x}$ = the standard error of a corresponding mean; n = the number of animals comprising the sample (number of determinations).

TABLE 4.—Comparison of blood-calcium levels in different classes of animals

Classes	Difference in means	σd^1
Yearling bulls vs. herd bulls.....	² 2.57	0.349
Yearling bulls vs. 2-year-old heifers.....	² 1.33	.287
Yearling bulls vs. aged cows.....	² 1.94	.344
Herd bulls vs. 2-year-old heifers.....	² 3.90	.302
Herd bulls vs. aged cows.....	² 3.51	.357
2-year-old heifers vs. aged cows.....	.39	.295

¹ σd = The standard error of an indicated difference.

² = Significance at the 1-percent level.

The complementary association of calcium and phosphorus in the blood is shown when the mean blood-calcium values (table 3) are compared with the mean blood-phosphorus values (table 1). For

the herd bulls, the blood-phosphorus value is significantly less, while the calcium content of the blood is significantly greater, than for the yearling bulls. The same tendency for these mineral contents of the blood to vary with age is shown in the results for aged cows and 2-year-old heifers. The tendency for blood phosphorus to diminish and blood calcium to increase with age seems to be more marked in the male than in the female. It should be remembered, however, that the blood-calcium level may not reflect the true picture of what is taking place; that is, a low calcium intake and the consequent withdrawal of calcium from the bone and other tissues for the blood. Hence, the calcium level of blood, when used as an index of the existing calcium picture, must be interpreted with caution.

A brief study of variabilities for the values of blood-calcium and phosphorus within the four classes of animals is shown in table 5.

TABLE 5.—*Variabilities of the blood-calcium and phosphorous levels in the different classes of animals*¹

Class	C. V. (Ca)	σ C. V. (Ca)	C. V. (P)	σ C. V. (P)
Yearling bulls	15.58	1.63	20.00	1.03
Herd bulls	10.21	1.40	16.93	1.50
2-year-old heifers	15.22	1.25	15.95	.93
Aged cows	19.01	1.87	22.43	1.43

¹ C. V. = Coefficient of variability ($100\bar{\sigma}_x$); σ C. V. = the standard error of the coefficient of variability.

The data presented in table 6 show the results of correlation studies upon samples from each homogeneous group under study.

TABLE 6.—*Correlation of the blood-phosphorous and calcium levels in the different classes of animals*¹

Class	r	σ r	n
Yearling bulls	−0.39	0.129	45
Herd bulls	−0.03	.192	27
2-year-old heifers	² −.25	.117	66
Aged cows	.01	.140	54

¹ r = Coefficient of correlation; σ r = the standard error of correlation; n = the number of animals comprising the sample (number of determinations). ² Significance at the 1-percent level.

The negative values for the correlation coefficient indicate the inverse association of calcium and phosphorous in the blood. The correlations for the herd bulls and for the aged cows are not significant, possibly owing to the fact that these classes of animals are heterogeneous as to age. This indicates that the calcium-phosphorus ratio is a variable concept depending on age and sex, as indicated by table 7.

TABLE 7.—*The blood-calcium and phosphorus ratio in different classes of animals.*¹

Class	\bar{x}	$\sqrt{\bar{x}}$
Yearling bulls	1.43	0.054
2-year-old heifers	1.60	.060
Aged cows	1.94	.090
Herd bulls	2.73	.083

¹ \bar{x} = Arithmetic mean average; $\sqrt{\bar{x}}$ = the standard error of a corresponding mean.

All differences between mean calcium-phosphorus ratios (table 7) are significant. There is no tendency for this ratio to be constant in a heterogeneous group of animals. That there is a strong tendency for blood phosphorus to diminish with age in both sexes is the principal conclusion.

SUMMARY

The normal blood levels of phosphorus and calcium for range Hereford yearling and herd bulls and for range Hereford heifers and aged cows have been determined. The blood levels reported are based on analyses of samples from 560 animals.

The normal levels for blood serum inorganic phosphorus in milligram percent were 7.30 ± 0.103 , 4.76 ± 0.099 , 5.07 ± 0.063 and, 4.89 ± 0.101 for yearling bulls, herd bulls, 2-year-old heifers, and aged cows, respectively.

The phosphorus level of blood was found to be greater in bulls than in cows. The quantity of phosphorus diminishes with age in Hereford range cattle, but this relationship with age is stronger in the male animal.

The normal levels for blood calcium in milligram percent were 10.46 ± 0.238 , 13.03 ± 0.256 , 9.13 ± 0.158 and 9.52 ± 0.249 for yearling bulls, herd bulls, 2-year-old heifers, and aged cows, respectively.

A complementary association of phosphorus and calcium has been found as shown by the fact that $r = 0.39 \pm 0.129$ for yearling bulls, -0.03 ± 0.192 for herd bulls, -0.25 ± 0.117 for 2-year-old heifers, and 0.01 ± 0.140 for aged cows.

The calcium-phosphorus ratio in homogeneous groups is a variable concept depending on age and sex.

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EFFECT OF MATURITY ON THE CHEMICAL COMPOSITION AND DIGESTIBILITY OF THE STEMS AND LEAVES OF SWEETCLOVER HAY¹

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INTRODUCTION

As a hay and pasture crop sweetclover is becoming increasingly important. Varietal tests have pointed to the Madrid (*Melilotus officinalis* (L.) Lam.) and to the Spanish (*M. alba* Desr.) varieties as excellent yielders under farming conditions in eastern Washington. Since tonnage yield alone is not a reliable criterion of value, however, studies of the chemical life history of the Madrid and of the digestibility of the Spanish sweetclovers were carried out in order to test the suitability of these two varieties for pasture and hay.

REVIEW OF LITERATURE

A brief review of the literature bearing on the composition and digestibility of sweetclover was given in a previous paper.² Hay of the Alpha 1 variety was found to contain 8.8 percent digestible crude protein and 46.13 percent total digestible nutrients in its first-year crop when cut at a height of about 36 inches, and 9.38 percent digestible crude protein and 47.18 percent total digestible nutrients in the second-year crop when cut for hay at the bud stage. Similar figures for the Common White variety were 10.21 percent digestible protein and 47.44 percent total digestible nutrients the first year, and 8.93 percent digestible protein and 41.94 percent digestible nutrients the second year.

PLAN OF INVESTIGATION

During the years 1939 and 1940 a study of the chemical life history of Madrid sweetclover was undertaken. Digestion experiments were also made with sheep fed samples of hay from first- and second-year Spanish sweetclover cut at definite stages of maturity. A height of approximately 36 inches seemed best for the first year, and the early

¹ Received for publication September 25, 1944. Published as Scientific Paper No. 621, College of Agriculture and Agricultural Experiment Station, State College of Washington.

² SOTOLA. DIGESTIBILITY OF NUTRIENTS IN FOUR VARIETIES OF SWEETCLOVER HAY. Jour. Agr. Res. 61: 887-891. 1940.

bud stage for the second year. Since field curing of sweetclover is usually accompanied by a large leaf loss, this study was designed to show what proportions of the digestible nutrients are found in the stems and leaves of the first and second year's growth. The plan provided for six separate digestion experiments in which stems, whole hay, and leaves of the first- and second-year crops were used separately during 10-day digestion experiments, each experiment being preceded by a 7-day preliminary feeding period.

METHODS OF SAMPLING AND ANALYSIS

CHEMICAL LIFE HISTORY OF MADRID SWEETCLOVER

Clippings of the new seeding were first taken when the plants were 7 inches high and thereafter at approximately 2-week intervals throughout the growing season. Different plants were selected each time and cut 1 inch above the ground line. Each clipping was made at approximately 9:00 a. m., and the samples were immediately taken to the laboratory, where they were air-dried, chopped, mixed, and smaller samples set aside for chemical studies. (Special precautions were taken to retain complete samples, avoiding leaf and other losses.)

The forage samples were subjected to the usual chemical analysis to obtain information as to the changes in the nutrient content that take place during the development of the plants.

The second spring all dead stalks of the previous year's growth were removed so as not to contaminate the new growth during the sampling operation.

DIGESTION EXPERIMENTS

Samples of 3,000 pounds each of the first- and second-year crop were carefully air-dried on canvas, and two-thirds of each sample was separated into stems and leaves. In separating the stems and leaves, all small petioles and blossoms were included with the leaves and all weeds were discarded. The samples were chopped into half-inch lengths and aliquots taken for chemical study. The daily feed requirement for each sheep was immediately weighed into paper bags to avoid discrepancies in dry-matter intake between trials arising from variations in humidity.

The detailed method of conducting digestion experiments with sheep by the use of metabolism cages has been previously described.³ Official methods⁴ of analysis were used for all chemical determinations.

EXPERIMENTAL RESULTS

CHEMICAL LIFE HISTORY OF MADRID SWEETCLOVER

Seedlings of Madrid sweetclover, 7 inches high, were sampled June 13, 1939. Sampling continued at intervals of about 2 weeks into October, at which time the plants were approximately 34 to 36 inches high.

³ SOTOLA, J. RELATION OF MATURITY TO THE NUTRITIVE VALUE OF FIRST, SECOND, AND THIRD CUTTINGS OF IRRIGATED ALFALFA. *JOUR. Agr. Res.* 35: 361-383, illus., 1927.

⁴ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 5, 757 pp., illus. Washington, D. C. 1940.

The first samples of the second year's growth were collected on April 30 when the plants were 5 inches high, and samplings continued every 2 weeks until July 9, at which time the plants were mature and approximately 73 inches in height. The relation of stems to leaves as affected by the height and maturity of the plant is shown in table 1.

TABLE 1.—*Relation of stems to leaves in air-dried Madrid sweetclover plants*

First-year crop				Second-year crop				
Date	Height above ground	Leaves air-dried	Stems air-dried	Date	Stage of maturity	Height above ground	Leaves air-dried	Stems air-dried
	Inches	Percent	Percent			Inches	Percent	Percent
June 13	7.0	28.47	71.53	Apr. 30		5.0	71.88	28.12
June 27	11.0	37.02	62.98	May 14		16.0	63.44	36.56
July 11	15.0	55.02	44.98	May 28		38.0	37.10	62.90
July 25	22.0	53.48	46.52	June 11	Early bud	66.0	28.48	71.52
Aug. 8	30.0	63.31	36.69	June 24	Bud	73.0	27.80	72.20
Aug. 22	36.5	63.45	36.55	July 9	Flower	73.0	26.56	73.44
Sept. 19	35.0	63.89	36.11					
Oct. 3	34.0	66.33	33.67					
Oct. 17	34.0	67.46	32.54					

The proportion of leaves steadily increased with the height and maturity of the plant during the first year. However, the same plants sampled during their second year's growth contained a high proportion of leaves early in the season and became stemmy as the season progressed.

The first-year crop on August 22, when the plants were 36.5 inches high, contained 63.45 percent of leaves, while the second-year crop on May 28, when the plants were 38 inches high, contained 37.1 percent of leaves. The mature plant of the first year's growth had a leaf-stem ratio of 1:0.48 as compared with a leaf-stem ratio of 1:2.76 for the mature plant of the second year's growth. The second-year crop matured earlier in the season than the first-year crop, and it was stemmier.

COMPOSITION OF THE DRY MATTER OF MADRID SWEETCLOVER

In 1939 at 7 inches high the fresh sweetclover contained 82.20 percent moisture; at 11 inches, 78.16 percent; at 15 inches, 68.00 percent; at 22 inches, 67.07 percent; at 30 inches, 62.33 percent; and at 35.5 inches, 53.00 percent. After this growth stage the moisture percentage remained fairly stationary for the remainder of the season. Almost identical moisture percentages were observed during the second year.

The chemical changes in maturing stems and whole plants of Madrid sweetclover (table 2) showed a progressive decrease in protein, ash, and fat, and an increase in crude fiber. The leaves showed some decrease in protein but the rate of decrease was lower.

In the second-year crop the protein content of the whole plants decreased sharply while a marked rise in crude fiber was observed. The leaves again changed at a much slower rate in regard to both nutrients, the changes being in the same direction.

TABLE 2.—Composition of the dry matter in Madrid sweetclover plants of the first- and second-year crop

STEMS											
First-year crop						Second-year crop					
Date	crude protein	Ash	Ether extract	Crude fiber	N-free extract	Date	Crude protein	Ash	Ether extract	Crude fiber	N-free extract
	Percent	Percent	Percent	Percent	Percent		Percent	Percent	Percent	Percent	Percent
June 13	10.76	11.43	4.45	21.02	52.34	Apr. 30	10.09	5.05	2.55	19.41	62.90
27	14.05	11.32	2.21	26.17	46.25	May 14	10.79	9.28	2.89	25.35	51.09
July 11	8.79	8.47	1.58	32.05	48.51	28	6.95	7.26	2.30	36.85	46.64
25	9.10	6.46	1.72	36.01	46.71	June 11	5.34	4.93	1.10	46.38	42.25
Aug. 8	8.44	5.50	1.35	44.70	40.01	24	4.34	3.85	.91	52.17	38.73
22	9.06	5.64	1.32	42.31	41.67	July 9	3.11	3.21	.82	53.70	39.16
Sept. 19	7.83	4.11	1.50	41.30	45.26						
Oct. 3	8.10	5.29	1.96	41.71	42.94						
17	7.73	4.10	1.44	41.78	44.95						

WHOLE PLANT											
June 13	23.07	12.84	4.18	11.54	48.37	Apr. 30	27.24	12.51	5.14	10.93	44.1
27	19.42	10.97	1.55	17.55	50.51	May 14	22.04	11.35	3.72	14.00	48.8
July 11	15.59	9.46	2.96	22.20	49.79	28	14.94	8.77	3.29	25.45	47.5
25	17.24	8.24	2.97	23.67	47.86	June 11	9.62	6.11	2.41	36.57	45.2
Aug. 8	14.40	7.07	2.67	31.84	44.02	24	8.86	5.03	2.10	39.47	44.5
22	13.36	6.98	2.65	31.62	45.39	July 9	8.35	4.89	1.88	42.99	41.8
Sept. 19	12.05	5.90	2.55	31.88	47.62						
Oct. 3	11.61	5.83	2.43	33.31	46.82						
17	10.68	6.05	2.59	31.01	49.67						

June 13	28.33	11.41	3.49	10.56	46.21	Apr. 30	30.71	12.83	4.92	7.79	43.75
27	25.07	11.47	3.27	8.76	51.43	May 14	23.70	11.90	5.38	7.60	51.42
July 11	22.95	11.57	4.84	10.17	50.47	28	22.67	11.63	5.74	8.54	51.42
25	26.36	10.35	4.45	9.75	49.09	June 11	23.22	10.24	4.80	10.49	51.25
Aug. 8	23.47	11.70	3.46	9.55	51.82	24	19.71	9.87	4.07	13.48	52.87
22	20.90	10.43	4.54	9.97	54.07	July 9	21.48	8.60	2.56	14.81	52.55
Sept. 19	19.77	9.07	3.76	8.63	58.77						
Oct. 3	18.19	9.38	5.05	9.65	57.73						
17	17.93	12.00	4.40	9.71	55.96						

CHEMICAL COMPOSITION OF SPANISH SWEETCLOVER

The chemical composition of the forage samples fed during the digestion experiments is shown in table 3. The level of protein was higher in the stems and leaves of the second- than of the first-year crop, as was true also of the crude fiber. The stems of the second-year crop harvested at the early-bud stage contained 42.31 percent of crude fiber.

The leaves of the first-year crop contained 3.286 percent calcium in contrast to 0.599 percent found in the stems. The second-year crop was lower in calcium but slightly higher in phosphorus than the first-year crop. The value for calcium in sweetclover hay compares very favorably with that for good grade alfalfa hay reported in an earlier paper.⁵

⁵ See footnote 2, p. 365.

TABLE 3.—*Chemical composition of Spanish sweetclover stems, whole plant, and leaves used in digestion trials with sheep*

FIRST-YEAR CROP (cut at 36 inches)

Sample	Moisture	Crude protein	Ash	Ether extract	Crude fiber	N-free extract	Calcium	Phosphorus
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Stems	12.90	6.05	4.07	1.07	36.41	39.50	0.599	0.230
Whole plant.....	10.76	9.95	6.64	1.18	26.98	44.49	1.734	.200
Leaves	17.39	15.40	9.22	2.67	9.11	46.21	3.286	.214

SECOND-YEAR CROP (cut at early bud stage)

Stems	7.04	10.25	6.73	1.30	42.31	32.37	0.582	0.372
Whole plant.....	9.11	16.58	10.20	2.04	30.41	31.66	1.285	.330
Leaves	8.19	24.88	12.62	3.28	14.88	36.15	2.073	.360

COEFFICIENTS OF APPARENT DIGESTIBILITY OF SPANISH SWEETCLOVER

The individual coefficients of apparent digestibility of Spanish sweetclover are shown in table 4. The sheep were fed at the same level of dry-matter intake, based upon the live weight of the sheep. Extreme care was taken to obtain an accurate partition of fecal and urinary nitrogen, and the nitrogen determinations were made on fresh feces in order to avoid any error that might arise from loss of ammonia in air-drying the feces. The feces, even in cool weather, were treated with dilute formaldehyde to prevent loss of ammonia from bacterial action.

TABLE 4.—*Coefficients of apparent digestibility of nutrients in the stems, leaves, and whole plant of Spanish sweetclover during its first and second year when harvested for hay and fed to weether lambs and yearlings*

STEMS

Lamb No.	First-year crop					Second-year crop				
	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract
180.....	56.0	55.7	28.4	41.8	71.3
188.....	51.7	49.7	38.9	38.4	67.4
190.....	50.5	47.2	24.7	35.6	68.6	50.6	55.5	58.9	43.2	63.0
196.....	48.4	47.0	29.9	32.0	67.3	54.3	57.9	51.5	46.0	66.9
283.....	40.9	55.2	54.5	42.1	61.8
Average.....	51.6	49.9	30.5	37.0	68.6	51.6	56.2	55.0	43.8	63.9

WHOLE PLANT

180.....	59.1	64.3	49.3	75.0	53.1	70.4	48.9	36.4	60.5
188.....	58.8	65.7	46.7	76.7
190.....	57.3	62.9	45.7	75.4	53.7	73.7	53.0	35.2	60.1
196.....	58.2	62.8	46.9	74.0	53.2	73.4	57.6	35.2	61.3
283.....	53.2	70.5	55.5	34.1	61.5
Average.....	58.4	63.9	47.2	75.3	53.2	72.0	53.8	34.7	60.0

LEAVES

180.....	63.7	75.2	5.0	41.4	77.3	61.6	75.5	38.7	41.6	69.7
188.....	66.1	77.5	8.0	45.1	79.2
190.....	62.9	75.3	9.8	31.9	77.5	59.3	77.4	42.8	32.2	66.9
196.....	64.8	76.2	5.1	47.7	78.3	64.4	79.0	43.2	43.7	72.0
283.....	64.4	78.5	43.6	44.2	73.8
Average.....	64.4	76.1	7.0	41.5	78.1	62.4	77.6	42.1	40.4	70.6

The wether lambs in these digestion experiments ranged from 37 to 59 kg. live weight and consumed a daily average of 15 to 19 gm. of dry hay per kilogram of live weight.

It may be observed in table 4 that there was practically no difference in the average digestibility of the dry matter in the stems of the first- and second-year crops. Likewise the dry matter of the whole hay of the first- and second-year crop and the dry matter of the leaves of the first- and second-year hay were quite similar. However, the dry matter of the leaves was almost 25 percent more digestible than that of the stems.

The average digestibility coefficient for crude fiber of the whole plant was 47.2 percent in the first-year crop and only 34.7 percent in the second-year crop, a difference of 36 percent in favor of the first-year crop.

The values of 76.1 and 77.6 as coefficients of digestibility of the protein in the leaves of the first- and second-year crop were very high, showing leaf protein on the average for both crops to be 45 percent more digestible than the protein of stems. Furthermore, leaves were much higher in protein than stems (table 3). The desirability of curing hay with a high leaf percentage is obvious from these figures.

DIGESTIBLE NUTRIENTS OF SPANISH SWEETCLOVER

The digestible nutrients in the stems, whole plant, and leaves of Spanish sweetclover are shown in table 5.

Assuming first-year sweetclover hay of the Spanish variety containing 52.59 percent of total digestible nutrients to have a value of 100 percent, the second-year crop would have a value of 84.1 percent on the basis of digestion experiments with sheep. On the basis of percentage composition, the stems of the first-year crop contained as much total digestible nutrients as the whole hay of the second-year crop, but only 25.8 percent as much digestible crude protein. Likewise, the leaves of the hay from the first-year crop contained only 60.7 percent as much digestible crude protein as the leaves of the second-year crop.

TABLE 5.--Digestible nutrients in the stems, whole plant, and leaves of Spanish sweetclover as determined with wether lambs for the first- and second-year crops, based on average coefficients of apparent digestibility

FIRST-YEAR CROP								
Sample	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Total digestible nutrients	Nutritive ratio ¹	Total digestible nutrient rating ¹
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>		
Stems.....	44.94	3.02	0.33	13.47	27.10	44.33	13.68	84.3
Whole plant.....	52.12	6.36	12.73	33.50	52.59	7.27	100.0
Leaves.....	53.20	11.72	.19	3.78	36.09	52.02	3.44	98.9
SECOND-YEAR CROP								
Stems.....	47.97	5.76	0.72	18.53	20.68	46.59	7.09	88.6
Whole plant.....	48.44	11.04	1.10	10.55	19.28	44.25	2.71	84.1
Leaves.....	57.29	19.31	1.38	6.01	25.52	53.94	1.79	102.6

¹ 1 to first-year whole hay 100 percent.

The second-year hay contained 64.8 percent more digestible crude protein than the first-year hay. According to previous work,⁶ sweetclover hay from plants of Common White sweetclover harvested at similar stages of maturity contained 10.21 and 47.44 percent of digestible protein and total digestible nutrients respectively in the first-year crop, with 8.93 and 41.94 percent in the second-year crop. Thus it is apparent from table 5 that the Spanish variety is superior both in the availability of its nutrients to sheep and its production of dry matter.

SUMMARY

Plants of Madrid sweetclover steadily increased in leaf percentage as they matured during the first year, but decreased at an even greater rate in leaf percentage during the second year. The plants decreased in protein, fat and ash and increased in crude fiber as they matured during the first year. As the second-year growth progressed the plants decreased sharply in protein and increased in crude fiber, much more so than during the first year. The leaves in both first- and second-year crops showed some decrease in protein, but the decrease was much less than that in the whole plant.

Digestion experiments in which sheep were fed Spanish sweetclover showed the first-year crop, cut for hay when 36 inches high, to contain 6.36 percent digestible crude protein and 52.59 percent total digestible nutrients. The second-year crop, more fibrous and stony, contained 11.94 percent digestible crude protein and 44.25 percent total digestible nutrients.

Leaf protein, on the average, was 45 percent more digestible than protein of the stems in both the first- and second-year crops of Spanish sweetclover.

⁶ See footnote 2, p. 365.

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